

Integration and Synthesis Summary for Amphibians

This Integration and Synthesis Summary includes our jeopardy analysis for any species that we or EPA determined will “likely be adversely affected” by the proposed action. Our jeopardy analysis of the proposed action’s impacts to listed species is split into three major factors: vulnerability, exposure, and toxicity. The tables below contain summaries of our rankings (high, medium, low) for vulnerability, exposure, and toxicity. Data and information used to determine each individual species’ rankings, including environmental baselines, cumulative effects, exposure information, and expected toxic effects for all species, and a template worksheet to show how rankings were assessed and combined are in Appendix E. Status of the species for each species can be found in Appendix B.

Vulnerability

For the amphibian species that we or EPA determined are “likely to be adversely affected” by the proposed action, we considered several factors for each species to determine the current vulnerability of that species to additional stressors. This effort allows us to consider whether a species’ current condition is stable, moving toward recovery or further decline. In general, we expect the species’ vulnerability to additional stressors to be higher if they are moving toward further decline than if they their condition is improving. We also identify which species are most (and least) susceptible to additional stressors in general based on information that could be surmised from species listing and recovery documents, or other sources as cited and considered in the Status section of this biological opinion.

Our assessment of vulnerability focuses on six factors: (1) the species listing status and recent 5-year status review recommendation (if available), (2) distribution, (3) number of populations, (4) species population trends, (5) if pesticides have been noted as a threat, and (6) impacts from activities associated with environmental baseline and cumulative effects. We obtained the information to create the vulnerability summary from the Status of the Species accounts (Appendix C), the overarching Environmental Baseline section of this Opinion, 5-year species status reviews, species recovery plans, species status assessments, and other sources containing the best available scientific information for the species.

We scored each of the six vulnerability components with high, medium, or low scores. We assigned a high vulnerability ranking to a species if all vulnerability components were scored as medium or high. We assigned a medium vulnerability ranking if a species’ scores were a mix of high, medium, and low (though exceptions were allowed for species that have a low status score or have an uplisting recommendation). We assigned a low vulnerability ranking to species with only low scores. Considerations regarding specific aspects of the species’ vulnerability or beyond what was included in the vulnerability ranking were applicable for some species depending on unique aspects of their life history. This information is reflected in the rationales for conclusion below.

Exposure

We anticipate amphibians can be exposed to carbaryl through contact with contaminated water in their habitats or via dietary exposure, depending on if they are a terrestrial species with an aquatic phase, or a fully aquatic species. We assume all carbaryl that is transported off-site, whether through spray drift or runoff, is likely to end up in local waterbodies, which may distribute carbaryl residues throughout the entire watershed. Carbaryl degrades quickly (i.e., within a few days) in aerobic aquatic habitats and as such is not likely to persist in waterbodies for long periods of time, be transported long distances in surface waters, or occur in groundwater sources. However, many amphibians may be exposed to carbaryl via multiple routes.

Exposure to Agricultural Uses

We characterize the expected level of exposure using overlaps between the species' ranges and agricultural areas where carbaryl is registered for use (i.e., overlap data), past carbaryl usage data (when available; the amount and location where carbaryl has been used in the past), any species-specific considerations such as life history information (e.g., habitat preferences, dispersal behavior), and existing protections or conservation actions (e.g., existing label measures, conservation measures from the action agency). Species with greater than 10% overlap between their range and agricultural carbaryl use sites are assigned a high overlap score, species with 5-10% overlap are assigned a medium overlap score, and species with less than 5% total overlap are assigned a low overlap score. In addition to range overlaps with carbaryl use sites, we considered past carbaryl usage data within a species' range to determine how much of a species' range we expect to be treated with carbaryl each year of the proposed action. Except where otherwise noted, usage data is provided by EPA applying data from their National and State Summary Use and Usage Matrix, as described in the Usage Analysis section of this biological opinion. Species that data indicate will have a large portion of their range (>10%) treated with carbaryl each year are assigned a high usage score. Species with 5-10% total usage are assigned a medium usage score, and species with less than 5% total usage are assigned a low usage score. Agricultural uses of carbaryl in the state of Hawai'i are no longer registered; however, agricultural uses are still registered for other island territories.

We determine the overall exposure ranking by qualitatively considering both the total overlap and total usage, as well as any additional exposure considerations that might modify the level of exposure likely to occur. When overlap and usage scores are the same, we assign the overall exposure ranking the same score (e.g., if both overlap and usage is high, the overall exposure ranking is high). In cases where overlap is high and usage is medium or when overlap is medium and usage is low, we use the overlap score as the overall exposure ranking to maintain conservative exposure assumptions. (As usage is a subset of overlap, the overlap score will always be greater than the usage score). In cases where overlap is high, but usage is low, we anticipate a large portion of the range may be treated over the duration of the proposed action even if only a small portion of the range is treated in any given year (particularly if the areas treated occur in different locations each year), leading to an overall exposure ranking of medium.

Past usage data for carbaryl is not available for species located on Pacific or Caribbean islands, including the Commonwealth of the Northern Mariana Islands, Guam, American Sāmoa, U.S. Virgin Islands, and Puerto Rico. Thus, in the absence of any additional exposure considerations for these species, our ranking is based on total overlap of carbaryl use sites for species that occur in these areas. For all species, where there are additional exposure considerations, we adjust the overall exposure ranking to reflect this additional information, as appropriate.

Exposure to Non-agricultural Uses

Carbaryl has several registered non-agricultural uses, including use sites within developed, open space developed, nurseries, rangeland, managed forests, and rights of way Use Data Layers (UDLs). Rights of way includes roadsides, and we refer to roadsides when applicable. In many cases, data provided by EPA indicate low to high levels of overlap between species' ranges and non-agricultural UDLs. However, UDLs for non-agricultural uses tend to be less defined than those for agricultural UDLs and may not accurately represent the actual footprint of these use sites on the landscape. As such, we assess exposure of species to non-agricultural uses of carbaryl in a qualitative manner, considering the life history of species, methods of application, carbaryl usage, and any existing conservation measures to reduce drift and runoff or otherwise limit exposure to species.

For most species, we anticipate that non-agricultural uses will not meaningfully add to the overall level of anticipated exposure considered in our analysis of agricultural uses and discuss each use in more detail in the *Overall Considerations for the Opinion* section of the Opinion. Briefly, we expect listed species are generally unlikely to be exposed to non-agricultural uses of carbaryl due to low levels of past usage and/or existing mitigation measures that are protective of listed species. Usage data summarized by the EPA indicate that all non-agricultural UDLs have very low levels of past usage (at most 2.5% treatable areas treated with carbaryl annually). Some use patterns, like rights of way, are particularly low usage areas, with less than 500 lbs of carbaryl applied nationally each year.

Additionally, based on application information, we anticipate carbaryl use in these UDLs are restricted to small treatment areas that are treated infrequently over long periods of time. Use patterns like forestry, rangeland, or rights of way may even be geographically restricted as available past usage data indicate carbaryl usage is only in certain areas of the country, such as the western conterminous United States. Available usage data from the U.S. Forest Service indicate that, over a five-year period (from 2016-2020), the Forest Service treated 322 acres of forests in California and 557 acres of forests across three Forest Service Regions (covering North Dakota, Montana, South Dakota, Idaho, Kansas, Nebraska, Colorado, Wyoming, Utah, and Nevada), with the majority of applications taking place in small areas (less than 1 acre in size). Similarly, usage data from the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS) show limited past carbaryl usage as well. From 2019-2023, APHIS treated 92,309 acres of rangeland in seven states (Arizona, Idaho, Montana, Nevada, Utah, Washington, Wyoming) and 25 counties. While this represents a large area overall, when

distributed across the areas within the seven states where usage occurs, we anticipate only a small percentage of any species' range is likely to be treated for this use pattern. Additionally, all but one of these applications were made using carbaryl bait, which we expect has a much lower risk profile as bait applications are not likely to cause off target exposures as there is no spray drift or contact exposure likely to occur.

Additionally, there are several existing conservation and mitigation measures for non-agricultural uses of carbaryl that will reduce the likelihood of exposure to listed species. For example, from the 2022 FIFRA Proposed Interim Decision and the 2024 NMFS biological opinion for carbaryl, residential treatments are limited to spot and crack treatments (defined as a 2 ft² area), crack-and-crevice treatment, or narrow perimeter bands around urban structures (from 1 inch to 6 feet). This limitation in application method renders off-site spray drift unlikely and greatly reduces the extent of area that can be treated in the developed and nurseries UDLs. Similarly, we anticipate all rangeland applications of carbaryl will be carried out in association with USDA APHIS as part of their grasshopper and Mormon cricket suppression program (USFWS 2024), which include many conservation measures that are meant to protect listed species from exposure. Examples of measures included a reduced agent area treatment strategy that minimizes the amount of pesticide applied within a treatment block, allowance of only one application per year, reduced application rates, minimized treatment area size within 500 feet and 1000 feet from listed species ranges for ground and aerial applications, respectively, and extended application buffers when applications are made near the listed species' habitat (e.g., up to 750 feet for some ground applications and up to a mile for some aerial applications).

To assess the likelihood of exposure to non-agricultural uses of carbaryl, we conducted a habitat assessment for each listed species, incorporating available information regarding habitat preferences, known occurrences, relevant life history traits or behaviors, as well as relevant available usage data (summarized in the above sections). For species whose habitat is known or presumed to occur in or adjacent to non-agricultural use sites, we consider, individually and qualitatively, the extent and manner of non-agricultural carbaryl usage within the species' range to generally determine whether a small, moderate, or large number of individuals are likely to be exposed and the expected level of adverse effects from non-agricultural exposure of carbaryl.

Conservation Measures

As part of the 2022 proposed interim decision for carbaryl, the technical registrants committed to a number of conservation measures for the protection of listed species, including a 48-hour rain restriction and mandatory 25-foot and 150-foot application buffers from aquatic habitats for all outdoor ground and aerial applications, respectively. We anticipate these measures will contribute to the protection of listed amphibian species by reducing the amount of carbaryl residues that is transported off use sites and into the habitat of listed species.

Additionally, an existing letter of concurrence issued by the Service to USDA APHIS regarding carbaryl use in their rangeland grasshopper and Mormon cricket suppression program requires

the implementation of numerous conservation measures for the protection of listed species. Amphibian mitigations for all species that are within the action area for the USDA-APHIS grasshopper and Mormon cricket consultation are the following: a 2500-foot buffer for all ultra-low volume applications of carbaryl and a 300-foot buffer for all ground applications of carbaryl. For carbaryl bait aerial applications all amphibians are protected by a 750-foot buffer and a 100-foot ground buffer. These specific buffers apply for the following species that fall in the action area for the USDA-APHIS consultation: California red-legged frog, California tiger salamander (Central California DPS), Chiricahua leopard frog, Dixie Valley toad, foot-hill yellow-legged frog (North Feather DPS), Jemez Mountains salamander, Oregon spotted frog, Sierra Nevada yellow-legged frog, Sonoran tiger salamander, and Wyoming toad. For the California red-legged frog, Chiricahua leopard frog, and the foot-hill yellow-legged frog (North Feather DPS), there are specific instructions on where the buffers are applied with respect to the habitat for the species such as applying the buffer to upland or riparian habitat within and between occupied or previously occupied (frog) sites that are located within one mile of the treatment area. For the remaining amphibians in this Opinion that are outside the action area for the grasshopper and Mormon cricket program, we anticipate there is a low likelihood of the need to apply these program measures as grasshopper and Mormon cricket populations do not reach the level where they need to be suppressed in these areas. However, we anticipate the buffers and other mitigation measures would be applied as discussed in the biological assessment, if there were a need to use carbaryl applications for this reason within the remaining amphibian species' habitats.

Toxicity

We characterize the expected toxic effect to species based on the anticipated level of direct and indirect¹ adverse effects to individuals. Our analysis of toxicity assumes individuals are exposed to carbaryl at levels estimated by EPA's environmental exposure modeling and is focused on determining the level of adverse effect expected to occur once exposure has taken place. Direct effects are based on the anticipated level of mortality and sublethal effects (e.g., reduced growth) likely to occur in exposed individuals. Indirect effects are based on the impact a listed species is likely to experience when the organisms they rely on, such as those that act as food or habitat resources, are exposed to carbaryl and experience adverse effects.

We consider estimated concentrations of carbaryl on the landscape or within the environment, and effects reported in available toxicity studies to determine the level of direct and indirect

¹ While our Opinion considers all consequences of the proposed action (per the definition of effects of the action at 50 CFR Part 402.02), the terms "direct" and "indirect" effects were used in EPA's BE, and are used in environmental risk assessment terminology in general, and do not have the same meaning as used in ESA regulations. As used in the effects analysis section, direct effects to species are those caused by the pesticide itself through dietary, dermal, or inhalation routes of exposure. Indirect effects occur when the pesticide acts on elements of the ecosystem that are required by the species, such as alterations to prey or shelter. Thus, in the effects analysis section, we may use these terms to link back to the analysis in EPA's BE.

adverse effects to listed species or critical habitat. Concentrations of carbaryl can vary greatly depending on where exposure takes place. For instance, exposures on or near use sites are at higher levels than exposures that occur in areas far away from use sites. Based on available toxicity data, we anticipate amphibians are highly sensitive to carbaryl at some estimated environmental concentrations and are likely to experience high levels of mortality, even in habitats that only accumulate low levels. We anticipate sublethal effects are likely to occur for amphibians at some estimated environmental concentrations as well, before the onset of mortality.

We anticipate species that rely on plant-based resources, such as algae and detritus for food or emergent aquatic vegetation as habitat, are not likely to experience any indirect adverse effects, as available toxicity data in plants indicate no reductions in plant survival or growth are likely to occur with carbaryl exposure. In contrast, species that may rely exclusively on other arthropods for food resources may experience high levels of indirect adverse effects as carbaryl exposure will likely reduce the abundance and availability of prey.

Concentrations of carbaryl can vary greatly among different regions and aquatic habitat types. We do not expect carbaryl to be persistent in the environment where it is able to dissipate or dilute quickly. Where carbaryl enters smaller streams or static waters (e.g., low flow/low volume waterbodies) from runoff or spray drift, we generally anticipate high levels of lethal and sublethal effects to individual amphibians where exposure occurs. In larger waterbodies (e.g., where concentrations may be lower due to dilution or other factors as described in the *Effects of the Action* Section of the Biological Opinion), we expect lower levels of lethal and sublethal effects to amphibians.

We determine the overall toxicity ranking for amphibians by qualitatively assessing both the expected levels of direct adverse effects (i.e., mortality) and indirect effects (i.e., prey loss). Given that mortality is the most adverse of direct effects to species, we assign a high toxicity score for direct adverse effects resulting in mortality. As mentioned previously, available toxicity data indicate amphibians are sensitive to carbaryl and may be exposed during the terrestrial phase via dietary exposure or during the aquatic phase via water as applicable to the species and are thus likely to die, even in habitats that only accumulate low levels.

Summary of Amphibians Conclusions

After reviewing the current status of the species, the environmental baseline for the action area, the effects of the proposed action, and the cumulative effects, it is our biological opinion that the registration of carbaryl, as proposed, is not likely to jeopardize the continued existence of the 45 amphibian species in this Appendix.

In our analysis below, some species that had the same or very similar rationales for their conclusions were grouped together, to increase efficiency and avoid repetition. Relevant information and data unique to each individual species was considered when assigning species to

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groups and incorporated into the rationales as appropriate. Species-specific information (e.g., environmental baseline, cumulative effects, status of the species, exposure, and toxicity) was considered for all species, including those species in the grouped analyses, and are presented in full in Appendices B and E. Species with rationales that did not fit in a group, or warranted a separate rationale because of their life history, conservation status, or other information indicated that effects could be different, have an individual discussion to provide additional explanation. This approach allowed us to streamline our discussion in this Opinion by avoiding repeating our findings when species in the respective groupings would be expected to be affected similarly. The use of these groupings, therefore, does not mean that our evaluation failed to evaluate each individual species. On the contrary, our process and analysis for each species remained the same, regardless of the format of the discussion presented below.

Species proposed for delisting

The following species is proposed for delisting (Table 1). While we present some specific information about the species in Table 1, we provide additional information on vulnerability (including environmental baseline and cumulative effects), exposure, and toxicity in Appendix E. The status of the species account can be found in Appendix B.

Table 1. Amphibian species proposed for delisting

Scientific Name	Common Name	Vulnerability Ranking	Exposure Ranking	Toxicity Ranking	Change in listing status	Determination
<i>Eleutherodactylus jasperi</i>	Golden coquí	-	Low	High	recommended delist - extinction	No Jeopardy

The golden coquí is endemic to Puerto Rico and its distribution was restricted to a small area south of the municipality of Cayey. The species was listed with low recovery potential and has a threatened status at present. However, the golden coquí has been recently recommended for delisting due to extinction based on findings in the most recent 5-Year Review (2022). When listed, the species was reported to occupy a total area of approximately 24 ha on mountain tops, from 700 to 850 m in elevation, at Cerro Avispa, Monte el Gato, and Sierra de Cayey. All known specimens were collected from bromeliads. They inhabited water-filled leaf axils of dense clusters of bromeliads growing on trees, rock edges, and on the ground. Most known habitat and critical habitat areas are found on private lands. Even though they were easily detected in the past, the species has not been detected in 40 years despite targeted surveys. All researchers that searched for the golden coquí after 1981 have used adequate and proven techniques for detection. However, their efforts have not yielded any observation of the species in its historical locations, neighboring locations, or new locations identified through habitat suitability models, strongly suggesting the golden coquí is extinct. There are no known extant populations of the golden coquí. Furthermore, much of the species' habitat has been modified. We did not assess risk and usage quantitatively for the golden coquí. Our analysis of this species is qualitative as we do not anticipate that exposure to carbaryl from agricultural or non-agricultural uses is reasonably certain to occur given the species' known distribution, the prior ease of detection when present, and the likelihood of extinction.

After reviewing the current status of the listed species, the environmental baseline for the action area, cumulative effects, and the effects of the action, it is our biological opinion that the registration of carbaryl is not expected to appreciably reduce the survival and recovery of the species in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of the golden coquí. As noted above, there are no known extant populations of the golden coquí as the species has not been found since the 1980s and is likely extinct. We did not assess risk and usage quantitatively for the golden coquí; however, we anticipate that exposure to carbaryl is very unlikely to occur given carbaryl's largely agricultural

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uses, the species' preferred forested mountain habitat and known distribution, and the likelihood of extinction.

Species with low exposure (informed by low overlap with agriculture)

The species in Table 2 are grouped because they have low concern of adverse effects due to low exposure as informed by low overlap between the species' range and agricultural land uses where carbaryl is registered for use. While we present some specific information about the species in Table 2 below, we provide additional information on vulnerability (including environmental baseline and cumulative effects), exposure, and toxicity in Appendix E. The status of the species accounts can be found in Appendix B.

Table 2. Amphibian species with low exposure, informed by low overlap with agriculture.

Scientific Name	Common Name	Vulnerability Ranking	Exposure Ranking	Toxicity Ranking	Total Agricultural Use Overlap (% range)	Determination
<i>Ambystoma cingulatum</i>	Frosted flatwoods salamander	High	Low	Medium	2.9	No Jeopardy
<i>Ambystoma mavortium stebbinsi</i>	Sonoran tiger salamander	Medium	Low	Medium	0.7	No Jeopardy
<i>Anaxyrus baxteri</i>	Wyoming toad	High	Low	Medium	0.8	No Jeopardy
<i>Anaxyrus canorus</i>	Yosemite toad	Medium	Low	Medium	0.2	No Jeopardy
<i>Batrachoseps aridus</i>	Desert slender salamander	High	Low	Medium	0.0	No Jeopardy
<i>Batrachoseps relictus</i>	Relictual slender salamander	High	Low	Medium	1.0	No Jeopardy
<i>Batrachoseps simatus</i>	Kern Canyon slender salamander	High	Low	Medium	1.0	No Jeopardy
<i>Cryptobranchus alleganiensis bishopi</i>	Ozark hellbender	High	Low	Low	3.9	No Jeopardy
<i>Eleutherodactylus cooki</i>	Guajón	High	Low	Medium	3.7	No Jeopardy
<i>Eurycea nana</i>	San Marcos salamander	High	Low	Low	3.9	No Jeopardy
<i>Eurycea sosorum</i>	Barton Springs salamander	High	Low	Low	1.8	No Jeopardy
<i>Eurycea waterlooensis</i>	Austin blind salamander	High	Low	Low	2.1	No Jeopardy
<i>Necturus alabamensis</i>	Black warrior (=Sipsey Fork) waterdog	High	Low	Low	3.8	No Jeopardy

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Scientific Name	Common Name	Vulnerability Ranking	Exposure Ranking	Toxicity Ranking	Total Agricultural Use Overlap (% range)	Determination
<i>Peltophryne lemur</i>	Puerto Rican crested toad	High	Low	Medium	3.9	No Jeopardy
<i>Phaeognathus hubrichti</i>	Red Hills salamander	Medium	Low	Medium	2.6	No Jeopardy
<i>Plethodon neomexicanus</i>	Jemez Mountains salamander	High	Low	Medium	0.5	No Jeopardy
<i>Plethodon nettingi</i>	Cheat Mountain salamander	Medium	Low	Medium	0.5	No Jeopardy
<i>Plethodon shenandoah</i>	Shenandoah salamander	High	Low	Medium	0.3	No Jeopardy
<i>Rana chiricahuensis</i>	Chiricahua leopard frog	Medium	Low	Medium	1.3	No Jeopardy
<i>Rana muscosa</i>	Mountain yellow-legged frog (Southern DPS)	High	Low	Medium	2.0	No Jeopardy
<i>Rana pretiosa</i>	Oregon spotted frog	Medium	Low	Low	4.3	No Jeopardy
<i>Rana sevosa</i>	Dusky gopher frog	High	Low	Medium	3.8	No Jeopardy
<i>Rana sierrae</i>	Sierra Nevada yellow-legged frog	High	Low	Medium	1.2	No Jeopardy

The species in Table 2 have high or medium vulnerability rankings, indicating that these species may be less robust in response to adverse effects from carbaryl than species with low vulnerability. These species have a medium or low toxicity ranking as mortality and some loss of prey abundance may occur if exposed to carbaryl. Some species, like the Wyoming toad and Puerto Rican crested toad, may occur at the edge of fields where carbaryl could be used, but we do not expect individuals will be exposed to levels of carbaryl that will cause measurable effects to growth, reproduction, or survival because the rain restriction measure and buffers already on the label reduce those exposures to levels below which we would observe these effects. Similarly, other species in this grouping may experience offsite runoff exposure, but we do not expect predicted concentrations of carbaryl in runoff will cause any measurable direct toxic effects to individuals that would adversely affect their growth, reproduction, or survival.

Thus, we anticipate, at most, a very small number of individuals are likely to be exposed to carbaryl. All the species in this group have low extent of overlap between agricultural use sites and their ranges (including associated off-site transport areas). Furthermore, the total agricultural overlap metric we use is a conservative estimate of exposure as it does not fully account for

redundancy between use site layers, assumes exposure is occurring in all possible overlapping areas, and does not consider information on past carbaryl usage. As such, we expect that exposure of these species to carbaryl will occur in an even smaller portion of the species' ranges. Where available, habitat preferences and data describing past carbaryl usage confirms this expectation. Thus, while these species' vulnerability and toxicity rankings may vary, we have high confidence that exposure will be limited to small portions of the species ranges from agricultural carbaryl use.

For non-agricultural uses of carbaryl, we qualitatively evaluated the potential for carbaryl exposure from use sites to individual species based on their preferred habitat and current known locations within the context of our expectation that overall, species will experience minimal exposure from non-agricultural carbaryl use sites (described in the Exposure section, above). Based on individual reviews of available life history information for each of the 23 species in Table 2, we expect that many of these species are unlikely to occur on, or near non-agricultural use sites of carbaryl. There are 11 species that we determined could occur on one or more non-agricultural use sites for which carbaryl is registered. However, for each of these species, we evaluated habitat use, occurrence information, and existing protections from recent Service documents and determined that exposure to non-agricultural carbaryl use is expected to be minimal based on the species' life histories, stressors, threats, and conservation measures in place as described above. For example, the guajón may be found in disturbed areas adjacent to rural roads, culverts, and aqueduct pump stations, but exposure is expected to be low as individuals spend a significant portion of their life underground within guajonales or caves formed from large boulders of granite rock formations (USFWS 2017). For species with significant overlap with rangeland use sites, we expect individuals are not likely to experience exposure to carbaryl from rangeland uses as USDA APHIS is required to implement existing conservation measures for species that occur where rangeland uses of carbaryl occur, as described above. For the Chiricahua leopard frog, ultra-low volume applications of carbaryl require a 2,500 foot aerial/300 foot ground buffer and are allowed along ephemeral and intermittent streams three miles from breeding sites and five miles along perennial water courses. For bait applications, a 750 foot aerial/100 foot ground buffer is required and will be applied along ephemeral and intermittent streams three miles from breeding sites and five miles along perennial water courses. Given the variety of usage data available, as well as existing conservation measures, for non-agricultural uses of carbaryl, we anticipate no more than a small number of individuals of each of the species in Table 2 will be exposed and experience adverse effects from non-agricultural uses of carbaryl.

The Puerto Rican crested toad may forage close to the agricultural areas near breeding ponds, and some are near agricultural areas where agrochemicals (i.e., pesticides, herbicides, and chemical fertilizers) may adversely affect the suitability of those breeding ponds (USFWS 2022a). However, estimated overlap of agricultural uses with the Puerto Rican crested toad range is low (3.9%), and many populations of the Puerto Rican crested toad are protected under conservation ownership.

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The San Marcos salamander, Barton Springs salamander, and Austin blind salamander are found in spring flows of the Edwards Aquifer. While recharge of these aquifer systems makes them susceptible to contaminants due to the porous nature of karst systems, carbaryl is not able to reach these springs because of its low persistence in water and the flow rates in the high flow waters where these salamanders are found is sufficient to dilute carbaryl to result in minimal exposure. We do not expect carbaryl to concentrate in the low flow/low volume waterbodies associated with these springs. In addition, there are several conservation activities that take place for the Edwards Aquifer including land acquisitions and conservation easements, water quality protection recommendations, regional water planning, the City of Austin's habitat conservation plan covering operation and maintenance of Barton Springs Pool and adjacent springs, as well as captive breeding (for the Barton Springs salamander) and water quality monitoring (USFWS 2016). In addition, all three salamanders' ranges have very little overlap with agriculture (1.8-3.9%).

The Red Hills salamander, Sonoran tiger salamander, and frosted flatwoods salamander spend significant portions of their life buried underground, in remote mountainous habitats, or in deep cave systems. For example, the Red Hills salamander is typically found in subterranean burrows. They fulfill much of their lifecycle near their burrows, prey on invertebrates and land snails inside the burrow and near burrow entrances, and do not inhabit agricultural areas (USFWS 2024). We revised the species' range for the frosted flatwoods salamander in 2023, after the submission of the final BE. The current range no longer includes many areas that may have historically been habitat and are no longer capable of supporting the species due to land use changes. Some habitat (i.e., mosaic of pine dominated flatwoods and seasonal wetlands) occurs near agricultural lands (USFWS 2020). However, the overlap with agricultural lands is low (2.9%) and is likely an overestimate from using the former, inaccurate range map for the BE.

The species' ranges for the Yosemite toad, mountain yellow-legged frog (southern DPS), Sierra Nevada yellow-legged frog, desert slender salamander, relictual slender salamander, Kern Canyon slender salamander, and Shenandoah salamander are primarily on protected or federal lands where we expect pesticide usage to be low (i.e., National Parks, National Forests), in addition to their ranges overlapping small areas of agricultural lands. Two species (i.e., relictual slender salamander, Kern Canyon slender salamander) also primarily occur in high elevation montane habitats (USFWS 2022b) that we expect will not be affected by carbaryl exposure.

In summary, the species in Table 2 have low exposure rankings as evidence by the low overlap of their ranges with agricultural land uses. The level of mortality will depend on the extent to which the species consumes contaminated dietary items, and due to low overlap with agricultural areas, we expect mortality to be low for these species. We anticipate reductions in the abundance of invertebrate prey species in terrestrial and aquatic areas. However, these reductions are not likely to occur throughout the entire species' ranges. We anticipate only a small number of individuals are likely to die or experience adverse effects to reproduction or growth from carbaryl exposure or reductions in prey abundance over the project duration. Therefore, we determine the overall risk of adverse effects to these species is low. After adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the

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survival and recovery of the species in Table 2 in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of these amphibian species.

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U.S. Fish and Wildlife Service. 2017. Guajón or Puerto Rican Demon (*Eleutherodactylus cooki*) 5-Year Review: Summary and Evaluation. Boquerón, Puerto Rico. 26 pp.

U.S. Fish and Wildlife Service. 2016. Barton Springs Salamander (*Eurycea sosorum*) Recovery Plan Amended to include Austin Blind Salamander (*Eurycea waterlooensis*). Albuquerque, New Mexico. 148 pp.

Species with low exposure (informed by low past usage from California Department of Pesticide Regulation data)

The species in Table 3 are grouped because they occur completely within California and have low exposure confirmed by low levels of past carbaryl usage within their ranges (% range treated), as informed by the California Department of Pesticide Regulation Pesticide Use Reporting (CalPUR) data from 2013-2022. While we present some specific information about the species in Table 3 below, we provide additional information on vulnerability (including environmental baseline and cumulative effects), exposure, and toxicity in Appendix E. The status of the species accounts can be found in Appendix B.

Table 3. Amphibian species with low exposure informed by low past usage from California Department of Pesticide Regulation, Pesticide Use Reporting Data.

Scientific Name	Common Name	Vulnerability Ranking	Exposure Ranking	Toxicity Ranking	% Range Treated (CalPUR)	Determination
<i>Ambystoma californiense</i>	California tiger salamander (Sonoma County DPS)	High	Low	Medium	0.0	No Jeopardy
<i>Ambystoma californiense</i>	California tiger salamander (Central California DPS)	High	Low	Medium	0.5	No Jeopardy
<i>Ambystoma californiense</i>	California tiger salamander (Santa Barbara County DPS)	High	Low	Medium	0.3	No Jeopardy
<i>Ambystoma macrodactylum croceum</i>	Santa Cruz long-toed salamander	High	Low	Medium	1.0	No Jeopardy
<i>Anaxyrus californicus</i>	Arroyo (=arroyo southwestern) toad	Medium	Low	Medium	0.0	No Jeopardy
<i>Rana boylei</i>	Foothill yellow-legged frog (Central Coast Range DPS)	High	Low	Medium	0.4	No Jeopardy
<i>Rana boylei</i>	Foothill yellow-legged frog (Coast Range DPS)	High	Low	Medium	0.1	No Jeopardy
<i>Rana boylei</i>	Foothill yellow-legged frog (Sierra Nevada DPS)	High	Low	Medium	0.1	No Jeopardy

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Scientific Name	Common Name	Vulnerability Ranking	Exposure Ranking	Toxicity Ranking	% Range Treated (CalPUR)	Determination
<i>Rana boylei</i>	Foothill yellow-legged frog (N Feather River DPS)	High	Low	Medium	0.0	No Jeopardy
<i>Rana draytonii</i>	California red-legged frog	Medium	Low	Medium	0.1	No Jeopardy
<i>Rana muscosa</i>	Mountain yellow-legged frog (Northern DPS)	High	Low	Medium	0.0	No Jeopardy

All the species in Table 3 have medium or high vulnerability rankings, indicating that they may not be able to withstand additional stressors in their environment, including mortality of individuals from carbaryl exposure. All species have medium toxicity rankings as we expect up to 24% of exposed individuals are likely to die. This number represents an upper bound of mortality if these amphibians consume only prey from a field treated with carbaryl or spend some of their lifecycle in small, low flowing waterbodies. We know from the life history of these species that level of mortality will depend on the extent to which the species consumes contaminated dietary items, which we expect to occur at some point over the course of the proposed action. We also anticipate reductions in the abundance of invertebrate prey species in low flow/low volume habitats, but reductions are not likely to occur throughout the entire species' range. Aquatic invertebrate prey is likely to be replenished from upstream sources for low flow/low volume waters.

While species in Table 3 have medium to high vulnerability and individuals may experience reductions in prey, we anticipate only a small number of individuals are likely to be exposed to carbaryl from agricultural use given that CalPUR data indicate low past usage within their ranges. While these species have relatively higher percent overlap between agricultural uses and their ranges than species in other tables, CalPUR carbaryl usage data indicates that very little carbaryl has been used from 2013-2022 within the sections where these species' ranges occur. Given that this usage reporting is mandated by the state of California and that these data are provided regularly at a relatively high spatial resolution, we have high confidence that only a small percent of the species' ranges is likely to be exposed to carbaryl. Where available, habitat preferences confirm this expectation. For example, the mountain yellow-legged frog (northern DPS), Santa Cruz long-toed salamander, and California red-legged frog occur in areas where we do not expect carbaryl use to occur (e.g., National Forests, subterranean habitats, protected lands). These species are unlikely to frequent non-agricultural use sites or agricultural use areas.

For non-agricultural uses of carbaryl, we anticipate that certain non-agricultural usage, such as that performed by professional commercial applicators, will be captured in the CalPUR data. In addition, we qualitatively evaluated the potential for carbaryl exposure from use sites to individual species based on their preferred habitat and current known locations within the

context of our expectation that overall, species will experience minimal exposure from non-agricultural carbaryl use sites (described in the Exposure section, above). Based on individual reviews of available life history information for each of the 11 species in Table 3, we expect that many of these species are unlikely to occur on, or near to non-agricultural use sites of carbaryl. There are four species that we determined could occur on forests, developed, open space developed, rangeland, and/or rights of way use sites. However, for each of these species, we evaluated habitat use, occurrence information, and existing protections from recent Service documents and determined that exposure to non-agricultural carbaryl use is expected to be minimal based on the species' life histories, stressors, threats, and conservation measures in place as described above. For example, developed and open space developed use sites have a significant overlap with the range of the Arroyo toad. However, Arroyo toads are breeding habitat specialists and need slow moving streams that are composed of sandy soils with sandy streamside terraces. Reproduction is dependent upon the availability of very shallow, still, or low-flow pools in which breeding, egg laying, and tadpole development occur, which is less likely to be found in developed or open space developed areas. Given available usage data, we anticipate no more than small numbers of individuals of each species listed in Table 3 will be exposed to carbaryl through non-agricultural uses and experience adverse effects (including death or sublethal impacts).

In summary, we expect no more than low levels of exposure to carbaryl or reductions in prey abundance for these species. Therefore, we determine, at most, a small number of individuals will die or experience effects to reproduction or growth from the proposed action, and the overall risk of adverse effects these species is low. After adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the survival and recovery of the species in Table 3 in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of these amphibian species.

Species with low exposure (informed by low past usage from USDA Census of Agriculture)

For the species in Table 4, we expect low exposure confirmed by low levels of past insecticide usage within their ranges (% range treated), as informed by the USDA's Census of Agriculture (CoA). While we present some specific information about the species in Table 4, we provide additional information on vulnerability (including environmental baseline and cumulative effects), exposure, and toxicity in Appendix E. The status of the species account can be found in Appendix B.

Table 4. Amphibian species with low exposure, informed by low past usage from USDA's Census of Agriculture (CoA)

Scientific Name	Common Name	Vulnerability Ranking	Exposure Ranking	Toxicity Ranking	% Range Treated (CoA)	Determination
<i>Cryptobranchus alleganiensis alleganiensis</i>	Eastern hellbender	Medium	Low	Low	0.5	No Jeopardy

The eastern hellbender (Missouri DPS) has a medium vulnerability ranking, indicating that they may not be able to withstand additional stressors in their environment, including mortality of individuals from carbaryl exposure. The eastern hellbender (Missouri DPS) has a low toxicity ranking because it inhabits and prefers highly oxygenated streams with unembedded boulder, cobble, and gravel substrates in high flowing, larger waterbodies where carbaryl is not likely to persist, thus we anticipate mortality and the potential for impacts to reproduction to be low. In addition, Census of Agriculture data indicates a low level of insecticide usage overall (0.5% of its range treated annually), and we anticipate only a small number of individuals are likely to experience exposure from agricultural uses of carbaryl.

For non-agricultural uses of carbaryl, we qualitatively evaluated the potential for carbaryl exposure from use sites to individual species based on their preferred habitat and current known locations within the context of our expectation that overall, species will experience minimal exposure from non-agricultural carbaryl use sites (described in the Exposure section, above). We expect eastern hellbenders to be exposed to carbaryl from developed, open space developed, and rights of way use sites. Available usage data indicate that there is a low level of carbaryl usage in developed and open space developed areas (less than 2.5% of treatable acres are likely to be treated with carbaryl annually across the country), indicating that there is a low likelihood of exposure to the species from these uses. Furthermore, we expect many carbaryl applications in developed areas will be limited to hand-held equipment for spot treatments that limit the amount of run-off that may enter nearby aquatic habitats where the hellbender may be found. Available usage data indicate very little carbaryl usage is likely to occur in rights of way, with less than 500 pounds of carbaryl applied to rights of way nationally each year. While this may result in a large treatment footprint if all rights of way usage were concentrated in one location or within the eastern hellbender's range, we expect this is highly unlikely to occur and rather expect rights

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of way usage is likely to be sporadic across the national landscape, with only small amounts, if any, used within the species' range. Given the available usage data, we anticipate no more than a small number of individual hellbenders will be exposed and experience mortality or sublethal adverse effects from non-agricultural carbaryl use.

Where exposure occurs, we anticipate reductions in the abundance of invertebrate prey species in low flow/low volume habitats, but reductions are not likely to occur throughout the entire species' range. Aquatic invertebrate prey is likely to be replenished from upstream sources for low flow/low volume waters. For high flow/volume waterbodies, where we expect the eastern hellbender to most likely occur, we expect low levels of mortality and low levels of indirect effects that result in effects to reproduction through loss of prey resources..

In summary, we expect low toxicity for the eastern hellbender (Missouri DPS) and no more than low levels of exposure to carbaryl for this species and its prey. Therefore, we determine a small number of individuals may be affected and the overall risk of adverse effects the eastern hellbender (Missouri DPS) is low. After adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the survival and recovery of the species in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of the eastern hellbender (Missouri DPS).

Species with low exposure (based on habitat characteristics)

The species in Table 5 occur in the Edwards Aquifer system, where we expect no more than low levels of carbaryl will accumulate and exposure will be low. While we present some specific information about the species in Table 3, we provide additional information on vulnerability (including environmental baseline and cumulative effects), exposure, and toxicity in Appendix E. The status of the species accounts can be found in Appendix B.

Table 5. Amphibian species with low exposure (based on the characteristics of their preferred habitat)

Scientific Name	Common Name	Vulnerability Ranking	Exposure Ranking	Toxicity Ranking	% Range Treated	Determination
<i>Eurycea chisholmensis</i>	Salado salamander	High	Low	Low	18.8	No Jeopardy
<i>Eurycea naufragia</i>	Georgetown salamander	High	Low	Low	1.6	No Jeopardy
<i>Eurycea rathbuni</i>	Texas blind salamander	High	Low	Low	1.8	No Jeopardy
<i>Eurycea tonkawae</i>	Jollyville Plateau salamander	High	Low	Low	3.3	No Jeopardy

The Salado salamander, Texas blind salamander, Georgetown salamander, and Jollyville Plateau salamander in Table 5 have high vulnerability rankings, indicating that they may be especially susceptible to species-level impacts from additional stressors in their environment, such as adverse effects to individuals from carbaryl exposure. Additionally, pesticides are noted as a threat to these species in their 5-Year Reviews or Recovery Plans. Available toxicity data indicate that the species would experience mortality in low flow/volume waterbodies and indirect effects through loss of prey if exposed.

These species are found in spring flows of the Edwards Aquifer. While recharge of these aquifer systems makes them susceptible to contaminants due to the porous nature of these karst systems, carbaryl is not likely to reach these springs because of its low persistence in water and high flow rate of waters where these salamanders are found that are expected to dilute carbaryl to minimal concentrations. We do not expect carbaryl to concentrate in the low flow/low volume waterbodies associated with these springs. In addition, there are several conservation activities that take place for the Edwards Aquifer including land acquisitions and conservation easements, water quality protection recommendations, regional water planning, the City of Austin's habitat conservation plan covering operation and maintenance of Barton Springs Pool and adjacent springs, and water quality monitoring. As such, we anticipate only a very small number of individuals, if any, are likely to be exposed to carbaryl and die or experience prey losses that impact survival, or reproduction from agricultural or non-agricultural uses.

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In summary, we anticipate the Edwards Aquifer where these four salamanders are found is not likely to accumulate more than low levels of carbaryl as we expect the majority of carbaryl residues will degrade before entering the aquifer. Thus, while individuals are likely to die if exposed and pesticides are noted as a threat to the species, we anticipate few, if any, individual Salado, Georgetown, Texas blind, or Jollyville Plateau salamanders are likely to experience exposure. We determine the overall risk of adverse effects of carbaryl to these species is low. After adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the survival and recovery of the species in Table 5 in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of the Salado, Georgetown, Texas blind, and Jollyville Plateau salamanders.

Species with Individual Integration and Synthesis Summaries

For the species in Table 6, our preliminary exposure and toxicity rankings indicate that the proposed action may result in high adverse effects. As such, we discuss each species in more detail in individual Integration and Synthesis summaries below. In some cases, we modified initial exposure and toxicity rankings due to additional information regarding exposure and effects for individual species, as described below. For species that had a jeopardy determination in the draft Opinion, EPA incorporated species-specific conservation measures that the registrants agreed to incorporate into the description of the action to minimize exposure to the species. When relevant, we retained our evaluation that led to our Preliminary Conclusion and the need for species-specific measures and added an updated Final Conclusion to reflect the impacts of these species-specific measures.

Additional information on vulnerability (including environmental baseline and cumulative effects), exposure, and toxicity can be found in Appendix E. The status of the species accounts can be found in Appendix B.

Table 6. Amphibians with moderate to high adverse effects anticipated from the proposed action. We addressed each species in individual Integration and Synthesis summaries.

Scientific Name	Common Name	Determination
<i>Bufo houstonensis</i>	Houston toad	No Jeopardy
<i>Necturus lewisi</i>	Neuse River waterdog	No Jeopardy
<i>Ambystoma bishopi</i>	Reticulated flatwoods salamander	No Jeopardy
<i>Anaxyrus williamsi</i>	Dixie Valley toad	No Jeopardy
<i>Eleutherodactylus juanariveroi</i>	Llanero coquí	No Jeopardy

Integration and Synthesis Summary: Houston toad

Scientific Name:	Common Name:	Entity ID:
<i>Bufo houstonensis</i>	Houston toad	190

Species Overview

In reviewing the status of the species, species' range (Figure 1), and the environmental baseline and cumulative effects for the action area, the Service has determined that the species' vulnerability is high. In our evaluation of the effects of the proposed action to the species, we determined there was high overlap of the action area with the species' range and high past usage of carbaryl within the species' range, indicating a high extent of exposure. We expect on-field exposure to occur rarely, and individuals that eat contaminated prey on-field or off-field will not die. We expected adults would experience high indirect effects through loss of prey because they rely on terrestrial invertebrates. We also expected sublethal effects through reduction in reproduction. We expected some mortality of aquatic phase life stages in low-flow/low-volume waterbodies. As such, we determined the risk of adverse effects to the species was high. We expected a large number of individuals would likely to experience adverse effects from the proposed action, and we expected species-level effects would occur.

Because of the effects described in our preliminary evaluation and conclusion, EPA and the applicant agreed to incorporate the species-specific conservation measures as part of the action. We now expect exposure for the Houston toad to be low. After incorporating conservation measures into the effects of the action, adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, and in light of the status of the species, we have determined the proposed action is not likely to appreciably reduce the survival and recovery of the Houston toad. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of the Houston toad. We discuss our rationale for this conclusion for the species in the sections below.

Species range

Based on range map dated: 1/26/2018; Wherever found; *States within the range:* TX

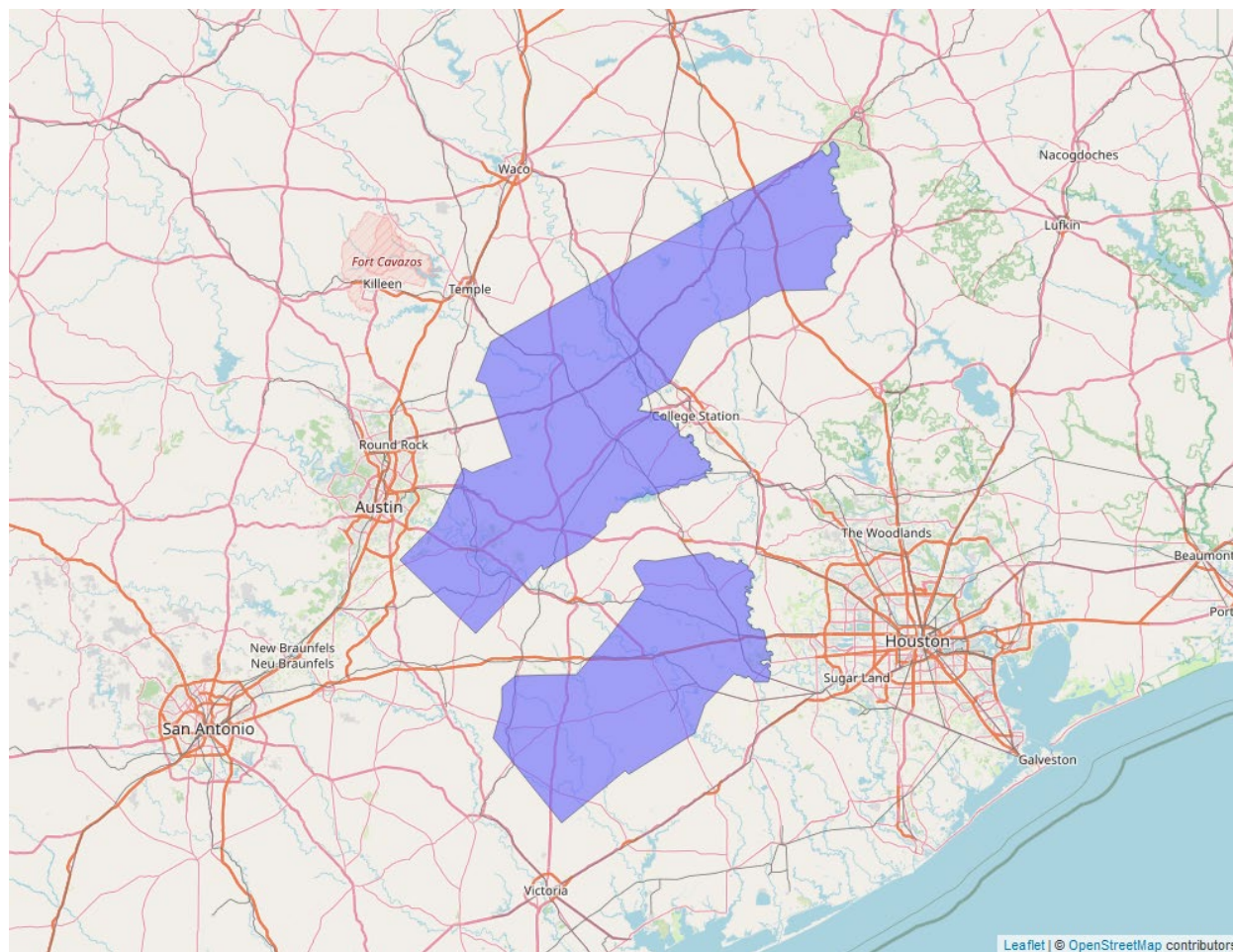


Figure 1. Range map of Houston toad (blue polygons). Range map accessed at <https://ecos.fws.gov/ecp/species/2206>.

Vulnerability

As mentioned above, vulnerability considers the present condition of the species to determine its vulnerability to additional stressors. Here, in making our jeopardy determination, vulnerability of the species is a function not only of its status, but also the environmental baseline and cumulative effects, as summarized below.

Summary of status

Listing status: Endangered

Most recent 5-Year Status Review recommendation: No change in Status

Most recently completed 5 Year Status Review: 7/6/2018

Distribution: Small, endemic, constrained, and/or isolated population(s)

Number of populations: Multiple populations (few)

Species trends: Declining population(s) - one or more populations declining

Pesticides noted in Service documents as a threat to the species: yes

Environmental Baseline/Cumulative Effects (EB/CE) Summary

Houston toads are a small, sedentary species with restricted distributions, specialized habitat niches, and narrow climatic tolerances. They are found in or near forested patches and canopy cover is a necessary component of its habitat. Adults require aquatic habitats to breed, typically standing or still water found in ephemeral ponds, wetlands, or other moist areas (e.g., ditches, lakes, puddles in roads, flooded pastures, potholes, streams, stock tanks, and permanent ponds). Tadpoles remain on the bottom of ponds during the day and along the pond's edge at night, and they feed on material attached to vegetation. Houston toads require terrestrial habitat for sheltering, feeding, and dispersal. They exhibit high site fidelity with occasional long-distance movements. Houston toads feed on insects and other invertebrates (e.g., carabids, other beetles, diptera, green lacewings, and small moths).

The historical range of the Houston toad encompassed portions of the following Texas counties: Austin, Bastrop, Burleson, Colorado, Fort Bend, Harris, Lavaca, Lee, Leon, Liberty, Milam, and Robertson. The current range is reduced to two bands across portions of nine counties in the Post Oak Savannah ecoregion (i.e., Austin, Bastrop, Burleson, Colorado, Lavaca, Lee, Leon, Milam, and Robertson). The species appears to be extirpated from Fort Bend, Harris, and Liberty counties. Majority of the current range is on private lands. Accurate populations estimates are difficult to ascertain due to detection difficulties (USFWS 2018), but we believe overall trends for Houston toad abundance are declining across its range. Only the Bastrop County population has been surveyed consistently from year to year since the 1970s. In the 1980s, surveyors reported observing 30 to 1,000 Houston toads per breeding pond in Bastrop County. Thereafter, estimates of 2,000 Houston toads in all of Bastrop County were reported. By 2003, the number of Houston toads in Bastrop County was estimated to be between 100 and 200 individuals. During the 2011 Houston toad breeding/survey season, only 12 Houston toads were detected from extensive surveys in Austin, Bastrop, Burleson, Colorado, Lavaca, Lee, and Milam counties, and limited survey attempts in Leon and Robertson counties. Houston toads may be extirpated from Lee County soon due to population trends and habitat loss observed there since 2000 (USFWS 2011). In 2007, the Houston Zoo established a head-starting program to raise Houston toads. In 2019, a captive assurance colony was established at the San Marcos Fish Hatchery. The fish hatchery produced and released about 10,000 eggs in 2020, their first breeding season. Captive propagation and headstarting since 2013 have resulted in population supplementation of Houston toads, principally at the Griffith League Ranch in Bastrop County, on the order of a million eggs per year since the program gained full efficiency in 2016. Results

have been promising, as captures of adult Houston toad at the Griffith League Ranch increased from 40 in 2016 and 63 in 2017 to 130 in 2018 and 126 in 2019 (USFWS 2022).

Houston toads disappeared from the Houston area (Harris, Fort Bend and Liberty counties) during the 1960-70s following an extended drought and the rapid urban expansion of the City of Houston. Habitat loss and fragmentation continues to occur throughout the species' range. Fire suppression, conversion to agricultural pastures, residential development, and artificial impoundments have contributed to a very different ecosystem and landscape than when the Houston toad was first described in 1953. Drought is an additional stressor and effects include desiccation, loss of breeding sites, and loss of eggs or tadpoles resulting from pond evaporation. Drought effects may be exacerbated due to other threats (e.g., habitat fragmentation and degradation). Predation by red imported fire ants is an ongoing threat to the species. The species' natural restrictions make them particularly vulnerable to the negative effects of human-induced changes that result in habitat loss, degradation, and fragmentation. The 1984 recovery plan mentions the herbicide atrazine as a potential threat to the species (USFWS 1984) and the 2022 revised recovery plan includes "[a]pplication of herbicides, pesticides, and fertilizers" from agriculture production as a contributor to Houston toad habitat loss and alteration. Conservation efforts have included development of Habitat Conservation Plans, Safe Harbor Agreements, and the purchase of land by Texas Parks and Wildlife Department for the conservation of the Houston toad. Results of captive propagation are still short-term, subject to frequent stochastic events (e.g., multiple catastrophic wildfires within designated critical habitat within the last 10 years), and do not address losses of habitat and the species' representation in other parts of the range (USFWS 2022).

Overall Vulnerability: High

Effects of the Action: Exposure

Overlap with Agricultural Use Sites

We expect 24.5% of the species range will overlap with carbaryl agricultural use sites or is likely to be exposed through off-site transport within the action area (Table 7). Up to 13.4% of the species' range overlaps with carbaryl use sites while 11% of the range occurs off-field and may be exposed to spray drift or runoff.

Usage

Based on past usage data, we anticipate up to 18.2% of the species' range will be treated with carbaryl annually.

Table 7. Overlap of carbaryl use sites with Houston toad range.

Use Layer	Use Site Overlap (% range)	Off-Site Overlap (% range)	Total Overlap (% range)	% Range Treated On-Site	% Range Treated Off-Site	% Total Range Treated
Alfalfa	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Citrus	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Corn²	5.6	3.4	8.9	1.8	1.1	2.9
Grapes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Other Crops	4	2.8	6.8	4	2.8	6.8
Other Grains	3.3	3.6	6.9	3.1	3.5	6.6
Other Orchards³	0.5	1.2	1.7	0.5	1.2	1.7
Other Row Crops	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Soybeans	0.6	0.5	1.1	0.2	0.2	0.4
Vegetables and Ground Fruit	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total	13.4	11	24.5	9.6	8.6	18.2

Additional Exposure Considerations

The Houston toad is found in areas with sandy soils and wooded areas (loblolly pine, and oaks) in nine counties east and northeast of Austin, Texas. Ephemeral ponds, rain pools, flooded field, and other shallow freshwater areas are used for breeding. Houston toads burrow into moist sand or hiding under rocks, leaf litter, logs, or in abandoned animal burrows in the forested areas to seek protection from winter cold (hibernation) and summer heat and drought (aestivation). Males call from shallow ravines, lakes, roadside ditches, ponds, temporary rain pools, flooded field, puddles, prairie potholes, and moist spots in residential areas. Breeding begins in January with egg-laying ranging from February to June. Although developmental rates depend on temperature and other factors eggs may hatch within seven days and tadpoles may remain in the breeding area for 40 to 80 days depending on environmental conditions. Toadlets may remain at the edge of the pond for seven to ten days. Young migrate away from breeding pools also similar routes of migrations used by adults. Adults may occupy upland areas and return to breeding areas during

² We expect corn and soybean use sites are highly redundant with each other and only use the higher of the two layers in our calculation of total percent overlap and total percent treated range.

³ We expect 'other orchards' and 'citrus' use sites are highly redundant with each other and only use the higher of the two layers in our calculation of total percent overlap and total percent treated range.

the breeding season. Thus, the Houston toad will migrate through agricultural, developed, and open space areas to arrive at breeding sites.

Non-agricultural Uses

Houston toads are not commonly found on non-agricultural areas but may disperse or migrate through developed areas or managed forests. Available data on past carbaryl usage in managed forests from the U.S. Forest Service from 2016-2020 indicate no carbaryl has been used by the Forest Service within the range of the species. Where applications have taken place, the majority of treatments have involved small areas (<1 acre). As such, we anticipate a low likelihood of carbaryl usage in the range, and that if usage did occur, exposure to the Houston toad would be minimal. Additionally, available usage data indicate that there is a low level of carbaryl usage in developed and open space developed areas (less than 2.5% of treatable acres are likely to be treated with carbaryl annually), indicating that there is a low likelihood of exposure to the species from these uses. Furthermore, we expect many carbaryl applications in developed areas will be limited to hand-held equipment for spot treatments that limit the amount of run-off that may enter nearby aquatic habitats where the Houston toad may be found. Available usage data indicate very little carbaryl usage is likely to occur in rights of way, with less than 500 pounds of carbaryl applied to rights of way nationally each year. While this may result in a large treatment footprint if all rights of way usage were concentrated in one location or within the Houston toad's range, we expect this is highly unlikely to occur and rather expect rights of way usage is likely to be sporadic across the national landscape, with only small amounts, if any, used within the species' range.

Exposure Summary

There is a high extent of overlap between agricultural use sites and the species' range. Based on past usage data, we expect a high level of agricultural usage within the range. Given that the extent of overlap is high, and that expected usage is high we expect a large number of individuals are likely to experience exposure from the proposed action.

Individuals may occur near non-agricultural use sites, including managed forests, developed, open space developed, and rights of way areas. However, based on limited occurrence in or adjacent to non-agricultural use sites of carbaryl, differences in application methods, low levels of usage and existing mitigation measures on product labels within these non-agricultural uses, we do not anticipate exposure from non-agricultural uses will result in the exposure of more than a small number of individuals.

Overall Exposure Ranking: High

Conservation Measures

Rain restriction: The carbaryl label has language designed to reduce the likelihood of pesticide runoff from use sites. This rain restriction language provides for a reduction in the concentration

of carbaryl in aquatic habitats by providing time for carbaryl to degrade before runoff into aquatic habitats can occur, decreasing exposure and risk to the Houston toad directly when in the aquatic phase as eggs or early metamorphs.

Aquatic habitat buffers: Application buffers are designed to reduce spray drift from entering sensitive non-target areas, thereby providing protection to aquatic species. While the exact amount of spray drift reduction depends on the physical traits of the aquatic ecosystem (e.g. flow rate, volume, etc.) as well as the application method, we can expect (based on AgDRIFT modeling) spray drift reductions with the spray drift mitigation practices already in place on the label. The carbaryl label has language to reduce the likelihood of pesticide spray drift from use sites specifically to nearby aquatic habitats. The label language states “Do not apply by ground equipment within 25 feet, or by air within 100 feet of lakes, reservoirs, rivers, estuaries, commercial fish ponds and natural, permanent streams, marshes or natural, permanent ponds.”

We anticipate that, in many cases, these buffers will significantly reduce exposure to the Houston toad and subsequent risk of direct effects and indirect effects to prey items.

Effects of the Action: Toxicity

Direct Effects

We do not expect Houston toads will die through dietary exposure of carbaryl. Adult Houston toads feed on a variety of insects and other invertebrates. Bragg (1960) reported that captive Houston toads favored many small to medium-sized carabids (ground beetles), several small beetles of unknown families, several dipteran (flies), green lacewings, and many types of small moths. However, we anticipate on-field foraging to be very limited as they do not prefer agricultural use sites or adjacent to fields. We expect low levels of carbaryl will occur in off-field food items compared to on-field food items.

Houston toads are known to travel through agricultural areas to arrive at breeding sites, but they do not contain suitable foraging habitat. We anticipate that foraging by the Houston toad could occur on use sites with low maximum application rates, and we do not expect direct effects to individuals from use of carbaryl at these application rates.

Aquatic phase:

EPA’s aquatic exposure modeling indicates that estimated environmental concentrations within the regions and aquatic habitats that the Houston toad occupies will likely be exposed to carbaryl at maximum concentrations of 1,553 µg/L, depending on the type of habitat. Mortality is not expected in large volume waterbodies but may occur in up to 3.7% of exposed individuals in low flow/low volume waterbodies where tadpoles and early metamorphs are found. We anticipate a reduction in fecundity and offspring survival in some low flow/low volume waterbodies as well. We do not expect any direct adverse effects from the consumption of algae and pollen by

Houston toad tadpoles. Once they leave the pond after metamorphosis, juvenile Houston toads feed on small invertebrates found on the forest floor.

Indirect Effects

Based on available life history information, we expect the Houston toad relies primarily on arthropods (particularly insects) for food resources. Based on available toxicity data, we expect individuals of these prey species will likely experience high levels of mortality with exposure to carbaryl, with greater mortality expected on-field than off-field due to lower carbaryl concentrations reaching off-field areas. As such, we expect there may be substantial reductions in the abundance of some invertebrate prey species throughout the species' range where use sites abut preferred habitats, indicating a high level of indirect adverse effects are likely to occur. However, invertebrates exhibit a range of sensitivities to carbaryl, and we expect exposure would reduce prey abundance, but not completely eliminate prey in exposed portions of the range.

Toxicity Summary

We expect a low level of direct adverse effects (i.e., sublethal) will occur to the Houston toad during the aquatic phase as eggs and early metamorphs. We expect Houston toads will primarily feed off-field and a small number that may feed on-field, but we do not anticipate any mortality from either of these exposure routes. We expect reduced fecundity is likely to occur at predicted exposure levels in small volume or low flow water bodies, and a high level of indirect effects are likely to occur to individuals from a high level of mortality to terrestrial invertebrates that act as the primary food resource for adults. As such, we determine the Houston toad has a medium toxicity ranking.

Overall Toxicity Ranking: Medium

Effects of the Action Summary

The Houston toad has a high exposure ranking. Based on agricultural land uses and past carbaryl usage data, we expect between 18.2-24.5% of the range may be treated annually over the duration of the proposed action depending how usage patterns change over time. This indicates that a large portion of the species' range is likely to be treated overall. As such, we expect a large number of individuals are likely to be exposed to carbaryl from agricultural uses. Based on available non-agricultural usage data, we do not anticipate more than a small number of individuals will be exposed through non-agricultural uses.

The Houston toad has a medium toxicity ranking. We do not anticipate mortality will occur on-field as a result of dietary exposure through the consumption of contaminated invertebrate food items, and we expect on-field exposure to be rare as agricultural areas are not suitable foraging habitat for the species. We expect a low level of mortality (3.6%) during the aquatic phase to tadpoles and early metamorphs in low flow/low volume waterbodies. In addition, we expect in

smaller, low flowing aquatic habitats, which comprises a large amount of the reproductive habitat of this species, and we expect effects to reproduction (e.g., reduced fecundity in adults and survival of offspring) in low flow/low volume waterbodies. We expect a high level of indirect adverse effects are likely to occur from the high level of terrestrial arthropod mortality with exposure at predicted concentrations of carbaryl.

Individuals may occur near non-agricultural use sites, including managed forests, developed, open space developed, and rights of way areas. However, based on their limited occurrence in or adjacent to non-agricultural use sites of carbaryl, differences in application methods, low levels of usage and existing mitigation measures on product labels, we do not anticipate non-agricultural uses will result in the exposure of more than a small number of individuals. Therefore, we anticipate low adverse effects from non-agricultural uses of carbaryl.

Given that we expect a high number of individuals are likely to be exposed and die from agricultural uses, indirect adverse effects through loss of terrestrial prey will be high, and some direct adverse effects are likely (especially to aquatic phases), we determine the overall risk of adverse effects to the species is high.

Preliminary Conclusion

The Houston toad has a high vulnerability ranking due to its endangered status, limited distribution, small population size, low juvenile survival rates, susceptibility to stochastic events, and anthropogenic threats to the species (e.g., continued degradation, fragmentation and loss of suitable aquatic and upland habitats from urbanization, invasive species, and agricultural impacts to habitat). Populations have continued to decline since at least the 1990s, and the isolated populations remaining are at risk from continued agricultural and development impacts. Pesticides were specifically mentioned in the species' environmental baseline and cumulative effects discussion above.

A high percentage (24.5%) of agricultural use sites overlap with the species' range in the action area, and past annual carbaryl usage for agriculture occurred on a large percentage of the range (18.2%). We do not expect non-agricultural uses to result in the exposure of more than a small number of individuals because levels of carbaryl usage for non-agricultural purposes are low and the toads are not commonly found on non-agricultural areas; they may disperse or migrate through developed areas or managed forests, but their primary habitats are not on these use sites. However, in our draft Opinion, before incorporating species-specific conservation measures, we anticipated that a large number of individuals would experience exposure from the action. While Houston toads are primarily a forest dwelling species, research demonstrates that the species can persist in a mosaic of landscapes, particularly in more arthropod-rich grasslands (Brown and Thomas 1982, Marsh 2016, Sirsi et al. 2020, Lamberts 2021). Houston toads are also highly mobile, particularly in the juvenile life stage (Vandewege et al. 2012), which is expected to increase the risk of exposure to the species (i.e., seasonally, most Houston toads exist as highly

mobile juveniles). It appears that agricultural conversion has limited the availability of suitable habitat through both structural change and chemical contamination.

In our draft Opinion, before incorporating species-specific conservation measures, we anticipated exposure to aquatic phases (i.e., egg and larval life stages) from runoff and spray drift and mortality of individuals at natal ponds across portions of the range where reproductive sites exist adjacent to agricultural use sites. In low flow/low volume waterbodies, we expected a low level of mortality (3.6%) to tadpoles and early metamorphs and effects to reproduction (i.e., reduced fecundity in adults and survival of offspring). Vulnerability of the aquatic life stage is high, and we anticipated toxic concentrations of carbaryl in the aquatic environment, particularly in smaller, low flowing habitats where tadpoles and early metamorphs are found. Once they leave the pond after metamorphosis, juvenile Houston toads feed on small invertebrates found on the forest floor that also will be affected by carbaryl exposure. We expected the greatest impact to the species to be through high losses of terrestrial prey from carbaryl exposure.

Without the conservation measures subsequently adopted as part of the action, as discussed below, we anticipated a large number of individual Houston toads would be exposed to carbaryl, and the impacts to the species would be through reductions in reproduction, mortality in aquatic habitats, and losses of invertebrate prey over the duration of the proposed action.

Final Conclusion (with Species-Specific Conservation Measures)

Because of the effects described in our preliminary conclusion above, EPA and the applicants agreed to incorporate the following measures as part of the action. Within the Pesticide Use Limitation Area (PULA) for the Houston toad:

- 1) For agricultural uses, carbaryl must be applied using a 105 foot buffer for ground applications and 160 foot buffer for airblast applications. Based on AgDRIFT modeling, the buffers will reduce spray drift from entering habitat for Houston toad by >95%. These buffer distances may be reduced using other measures identified as equivalent mitigations (i.e., reducing spray drift by similar magnitude) as specified in EPA's Draft Insecticide Strategy and as described in Appendix A-1 of this Opinion.*
- 2) Applicators need 3 points of mitigation as outlined in EPA's Draft Insecticide Strategy. This will reduce carbaryl loads in the habitat of the Houston toad by an order of magnitude (i.e., a 10-fold reduction).*

The PULA for the Houston toad will be developed as described in the Description of the Proposed Action section of the main Opinion and Appendix A-1. EPA is currently considering public comments received on the Draft Insecticide Strategy. If additional mitigation options become available during finalization of the Insecticide Strategy or in the future, this might warrant re-initiation to incorporate those measures into the action (i.e., additional options and mitigations for end users). In that case, EPA will provide documentation that these measures provide equivalent conservation for listed species, including reduction in off-site transport. Upon

confirmation by the Service, those options will be added to the acceptable mitigations listed for end users of carbaryl.

After incorporating the specific conservation measures above, we expect exposure for the Houston toad to be low. As such, we anticipate low numbers of individuals of this species will be adversely impacted. We anticipate loss of prey on agricultural fields adjacent to Houston toad habitat will incrementally reduce prey availability but at much reduced levels. Similarly, while direct exposure from consumption of contaminated prey and aquatic exposure through spray drift and runoff into breeding sites at the periphery of forested habitats is possible, we anticipate that, with the measures described above, these pathways of exposure will be greatly limited and result in the exposure of very low numbers of individuals or their prey over the course of the proposed action, leading to mortality of a very small number of individuals. After reviewing the current status of the species, environmental baseline for the action area, cumulative effects, and effects of the proposed action (including the species-specific conservation measures that are now incorporated into the proposed action), we have determined the proposed action is not likely to appreciably reduce the survival and recovery of the Houston toad. Thus, it is our biological opinion that the registration of carbaryl, as proposed, is not likely to jeopardize the continued existence of the Houston toad.

References

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- Marsh, M. J. L. 2016. Effects of red imported fire ants (*Solenopsis invicta*) on juvenile Houston toads (*Bufo houstonensis*) in a coastal prairie grassland. Report, Master of Science, Texas State University. 38 pp.
- Sirsi, S. M. J. Marsh, and M. R. J. Forstner. 2020. Evaluating the effects of red imported fire ants (*Solenopsis invicta*) on juvenile Houston Toads (*Bufo* [= *Anaxyrus*] *houstonensis*) in Colorado County, TX. *PeerJ* 10.7717/peerj.8480.
- U.S. Fish and Wildlife Service. 2022. Revised Recovery Plan for the Houston Toad (*Anaxyrus* [formerly *Bufo*] *houstonensis*). Albuquerque, New Mexico. 78 pp.
- U.S. Fish and Wildlife Service. 2018. 5-year Review: Houston toad (*Anaxyrus* [formerly *Bufo*] *houstonensis*). Austin, Texas. 1 pp.

Appendix C-A1. Amphibians: Integration and Synthesis Summaries

U.S. Fish and Wildlife Service. 2011. Houston Toad (*Bufo houstonensis*) 5-year Review: Summary and Evaluation. Austin, Texas. 22 pp.

U.S. Fish and Wildlife Service. 1984. Houston Toad Recovery Plan. Albuquerque, New Mexico. 73 pp.+iii.

Vandewege, M. W., D. J. Brown, and M. R. J. Forstner. 2012. *Bufo* (= *Anaxyrus*) *houstonensis* (Houston Toad). Headstart Juvenile Dispersal. Herpetological Review 43(1): 118.

Integration and Synthesis Summary: Neuse River waterdog

Scientific Name:	Common Name:	Entity ID:
<i>Necturus lewisi</i>	Neuse River waterdog	2932

Species Overview

In reviewing the status of the species, species' range (Figure 2), and the environmental baseline and cumulative effects for the action area, the Service has determined that the species' vulnerability is high. In our evaluation of the effects of the proposed action to the species, we determine there is high overlap of the action area with the species' range and high past usage of carbaryl within the species' range, indicating a high extent of exposure. Though we do not expect exposed adults will die in high flow waterbodies where the species is primarily found, the few that breed in low flow/volume waterbodies may experience reduced fecundity and offspring survival. The species will experience low indirect effects through loss of prey because they are aquatic prey generalists. As such, we determine the risk of adverse effects to the species is low. We expect a small number of individuals are likely to experience adverse effects from the proposed action, and we do not expect species-level effects will occur. After adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the survival and recovery of the species in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of the Neuse River waterdog. We discuss our rationale for this conclusion for the species in the sections below.

Species range

Based on range map dated: 10/10/2018; Wherever found; *States within the range:* NC

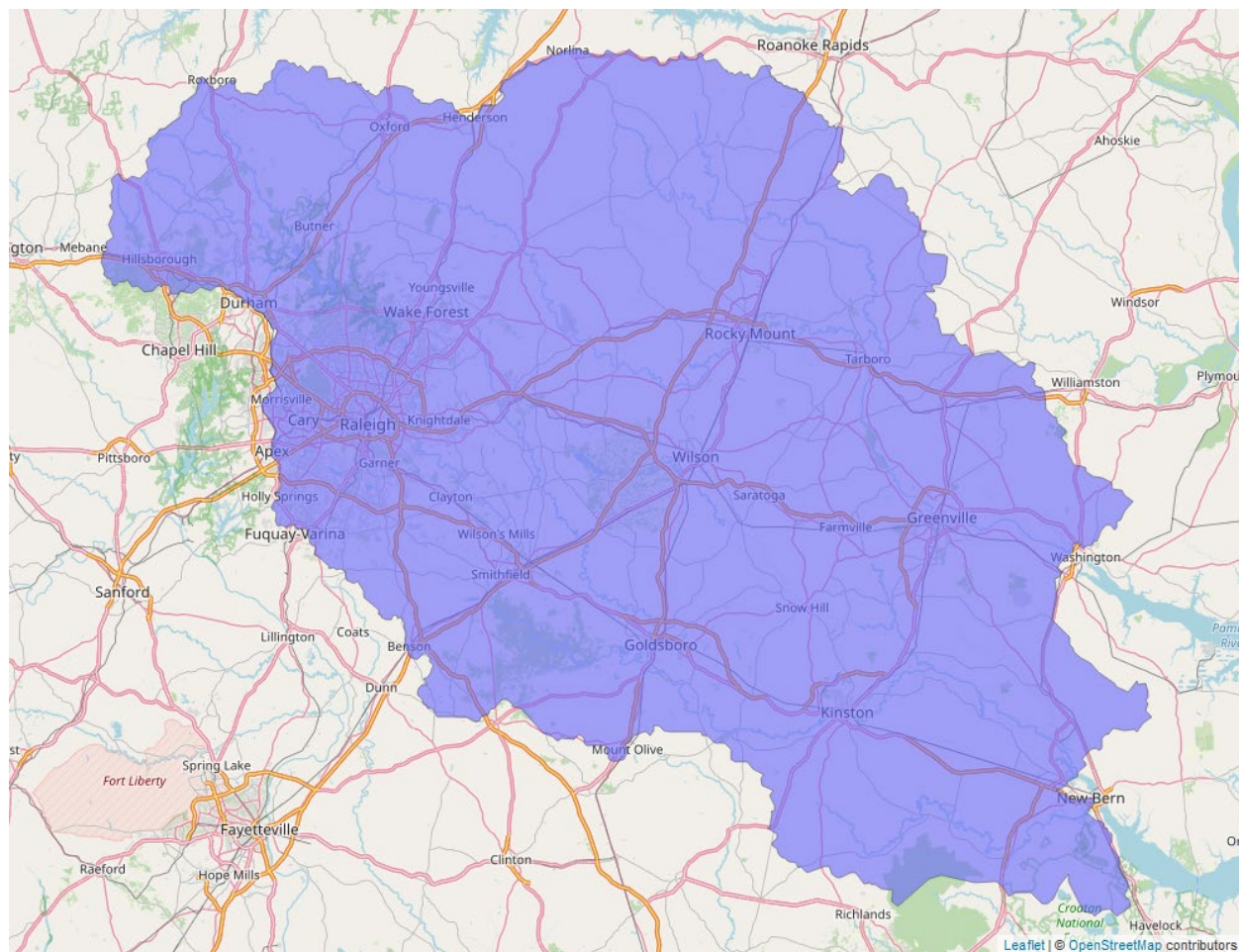


Figure 2. Range map of Neuse River waterdog (blue polygons). Range map accessed at <https://ecos.fws.gov/ecp/species/6772>.

Vulnerability

As mentioned above, vulnerability considers the present condition of the species to determine its vulnerability to additional stressors. Here, in making our jeopardy determination, vulnerability of the species is a function not only of its status, but also the environmental baseline and cumulative effects, as summarized below.

Summary of status

Listing status: Threatened

Most recent 5 Year Status Review recommendation: N/A

Most recently completed 5 Year Status Review: N/A

Distribution: Small, endemic, constrained, and/or isolated population(s)

Number of populations: Multiple populations (few)

Species trends: Declining population(s) - one or more populations declining

Pesticides noted in Service documents as a threat to the species: yes

Environmental Baseline/Cumulative Effects (EB/CE) Summary

The Neuse River waterdog is a permanently aquatic salamander species endemic to the Tar-Pamlico and Neuse River drainages in North Carolina. The species occurs in riffles, runs, and pools in medium to large streams and rivers with moderate gradient in both the Piedmont and Coastal Plain physiographic regions. Waterdogs prefer clean water with permanent flow and are not tolerant of siltation and turbidity. Benthic critters such as the waterdog have disproportionate rates of imperilment and extirpation because stream bottoms are often the first habitats affected by pollution. The Neuse River waterdog has declined in abundance and distribution and many remaining populations are fragmented (USFWS 2021a). Since the 2018 SSA analyses (USFWS 2021a), survey and research efforts have led to documentation of Neuse River waterdogs in places they were believed to be extirpated. The species was found in 37 HUC-10s between 2011-2022; 338 of 430 were added since 2018. As of 2023, the Neuse River waterdog has 3 populations: Trent, Neuse (8 subpopulations), and Tar-Pamlico (5 subpopulations) (USFWS 2023). The one population predicted to remain extant (Tar) is expected to be characterized by low occupancy and abundance in the future (USFWS 2021a).

The Neuse River waterdog faces a variety of risks from declines in water quality, loss of stream flow, riparian and instream fragmentation, deterioration of instream habitats, invasive species (i.e., red swamp crayfish (*Procambarus clarkii*), flathead catfish (*Pylodictis olivaris*), and hydrilla (*Hydrilla verticillata*)). These risks, which are expected to be exacerbated by urbanization and climate change, were important factors in our assessment of the future viability of the Neuse River waterdog. Streams with urbanized or agriculturally dominated riparian corridors are subject to increased sediment-loading from unstable banks and/or impervious surface run-off, resulting in less suitable in-stream habitat for waterdogs as compared to habitat with forested corridors. Agricultural pesticide use can have detrimental effects, and studies have shown the species to have low to moderate levels of pesticide contamination from a variety of sources, including insect control. The human population in the southeast has increased annually by 37.6% since 2000 and we expect additional growth in the future. With human population growth, we also expect additional urban development that could result in mortality or habitat loss for the Neuse River waterdog. Climate change has already begun to affect the watersheds where Neuse River Waterdog occurs, resulting in higher air temperatures, increased evaporation, and altered precipitation patterns such that water levels range-wide have reached historic lows, which put the populations at elevated risk for habitat loss, especially in the headwater areas. We expect other threats to the waterdog, including water quality issues, loss of stream flow, fragmentation,

and general habitat loss to be exacerbated by increased development and climate change (USFWS 2021a).

Overall Vulnerability: High

Effects of the Action: Exposure

Overlap

We do not expect listed aquatic species will occur on-field, and thus expect exposure will only result from off-field transport via spray drift or runoff. Given that the ranges for listed aquatic species are generally delineated using the relevant HUC 12 watersheds, we anticipate that all residues that leave use sites will be collected in the waterbodies within the species range where individuals occur regardless of how residues leave treated sites or where in the range they are deposited. As such, we do not extend overlap metrics off-field as this will not functionally change the expected exposures that listed aquatic species are likely to experience. We expect up to 34.7% of the species range will contain use sites (Table 8).

Usage

Past usage data indicate that up to 11.3% of the species' range has been treated with carbaryl annually. Use layers with the highest anticipated usage include vegetables and ground fruit and other crops at annual rates of 6% and 12%, respectively.

Table 8. Overlap of carbaryl use sites with Neuse River waterdog.

Use Layer	Use Site Overlap (% range)	% Range Treated On-Site
Alfalfa	<0.1	<0.1
Citrus	0	0
Corn	11	1.1
Grapes	<0.1	<0.1
Other Crops	4	4
Other Grains	0.5	<0.1
Other Orchards	<0.1	<0.1
Other Row Crops	5.6	0.5
Soybeans	21	3.2
Vegetables and Ground Fruit	3.5	3.5
Total	34.7	11.3

Additional Exposure Considerations

Neuse River waterdogs breed once per year, with mating in the fall/winter and spawning in the spring. During the spring (May-June), females will lay a clutch of ~25-90 eggs in a rudimentary nest, under large rocks in moderate currents. Ashton (1985) noted that nest sites are guarded by females and are often found under large bedrock outcrops or large boulders with sand and gravel beneath them, often placed there by the waterdogs (USFWS 2018).

Non-agricultural Uses

The Neuse River waterdog may be exposed to carbaryl run-off or spray drift through the non-agricultural uses for applications within developed areas or rights of way. However, available data on past non-agricultural usage indicate that very little insecticides, in general, are applied to utility rights of way nationwide, indicating that there is a low likelihood of exposure to the Neuse River waterdog. Additionally, available usage data indicate that there is a low level of carbaryl usage in developed and open space developed areas (less than 2.5% of treatable acres are likely to be treated with carbaryl annually). Furthermore, we expect many carbaryl applications in developed areas will be limited to hand-held equipment for spot treatments that limits the amount of run-off that may enter nearby aquatic habitats where the Neuse River waterdog may be found. Available usage data indicate very little carbaryl usage is likely to occur in rights of way, with less than 500 pounds of carbaryl applied to roadways nationally each year. While this may result in a large treatment footprint if all rights of way usage were concentrated in one location or within the Neuse River water dog range, we expect this is highly unlikely to occur and rather expect rights of way usage is likely to be sporadic across the national landscape, with only small amounts, if any, used within the species' range. As such, we do not expect non-agricultural uses will result in the exposure of more than a small number of individuals.

Exposure Summary

There is a high extent of overlap between agricultural use sites and the species' range. Based on past usage data, we expect a high level of agricultural usage within the range. Given that the extent of overlap is high, and that expected usage is high we expect a large number of individuals are likely to experience exposure from the proposed action.

Individuals may occur near non-agricultural use sites, including developed, open space developed, and rights of way areas. However, based on the low likelihood of usage within these non-agricultural uses, we do not anticipate exposure from non-agricultural uses will result in exposure to more than a small number of individuals.

Overall Exposure Ranking: High

Conservation Measures

Rain restriction: The carbaryl label has language designed to reduce the likelihood of pesticide runoff from use sites. This rain restriction language provides for a reduction in the concentration of carbaryl in aquatic habitats by providing time for carbaryl to degrade before runoff into aquatic habitats can occur, decreasing exposure and risk to the Neuse River waterdog directly.

Aquatic habitat buffers: Application buffers are designed to reduce spray drift from entering sensitive non-target areas, thereby providing protection to aquatic species. While the exact amount of spray drift reduction depends on the physical traits of the aquatic ecosystem (e.g. flow rate, volume, etc.) as well as the application method, we can expect (based on AgDRIFT modeling) spray drift reductions with the spray drift mitigation practices already in place on the label. The carbaryl label has language to reduce the likelihood of pesticide spray drift from use sites specifically to nearby aquatic habitats. The label language states “Do not apply by ground equipment within 25 feet, or by air within 100 feet of lakes, reservoirs, rivers, estuaries, commercial fish ponds and natural, permanent streams, marshes or natural, permanent ponds.”

We anticipate that, in many cases, these buffers will significantly reduce exposure to the Neuse River water dog and subsequent risk of direct effects and indirect effects to prey items.

Effects of the Action: Toxicity

Direct Effects

EPA’s aquatic exposure modeling indicates that EECs within the region and aquatic habitats that the Neuse River waterdog occupies will likely be exposed to carbaryl at concentrations up to 867 µg/L, depending on the type of habitat. These EECs encompass exposure expected from all uses, including both agricultural and non-agricultural. Mortality is not expected in high flow waterbodies and may occur in up to 0.17% of exposed individuals in low flow/low volume waterbodies. The Neuse River water dog prefers riffles, runs, and pools in medium to large streams and rivers with moderate gradient such as streams wider than 15m, although some have been observed in smaller creeks deeper than 100 cm, and with a main channel flow rate greater than 10cm/sec (USFWS 2021), so it may be found in both high flow waterbodies and low flow /low volume waterbodies. We anticipate a reduction in fecundity and offspring survival in some low flow/low volume waterbodies within the range of the Neuse River waterdog. However, breeding and nesting most likely occur in water bodies with moderate current protected under large boulders or bedrock outcrops below the water surface where EECs are likely to be lower than what would impact reproduction.

Indirect Effects

The Neuse River waterdog can consume invertebrate species as a food resource. Available toxicity data indicate that invertebrate species, particularly arthropods, are sensitive to carbaryl

and are likely to die with exposure to carbaryl at the predicted environmental concentrations. As such, we anticipate indirect effects to the species through the loss of prey resources is likely. However, we do not expect all invertebrate species will be equally sensitive to carbaryl exposure. Abundance of some invertebrate species may be reduced while other species may not exhibit as large of a reduction in abundance. In addition, we expect some reductions in zooplankton from carbaryl exposure but based on carbaryl's low persistence in water and planktonic drift, we anticipate any localized reductions in zooplankton as a food source will be quickly replenished by upstream sources. Given that available life history information available for the Neuse River waterdog indicates it is an invertebrate prey generalist, we anticipate individuals are likely more robust to temporary losses of certain invertebrate prey species as they can likely switch to use other species whose abundance is not as greatly reduced as they may have less inherent sensitivity to carbaryl. As such, we anticipate a temporary loss of certain invertebrate prey species will result in no more than low levels of adverse indirect effect to the Neuse River waterdog.

Toxicity Summary

Based on the predicted environmental concentrations of carbaryl within the aquatic habitats that the Neuse River waterdog is found, we expect there will be a low level of mortality. While some effects to reproduction are anticipated in low flow or low volume water bodies, the Neuse River water dog does not prefer these types of habitats for breeding and nesting. We anticipate a temporary low level of indirect effects to invertebrate prey. As such, we anticipate the species has a low toxicity ranking.

Overall Toxicity Ranking: Low

Effects of the Action Summary

The Neuse River waterdog has a high exposure ranking. There is a large presence of agricultural carbaryl use sites within the species' range (60.8% total overlap) and a high level of anticipated agricultural usage rate within the range (up to 23.8% of the range treated annually). As such, we expect a large number of individuals are likely to experience exposure. Individuals may occur near non-agricultural use sites, including managed forests, developed, open space developed, and rights of way areas. Based on available non-agricultural usage data, we do not anticipate more than a small number of individuals will be exposed through non-agricultural uses.

The Neuse River waterdog has a low toxicity ranking. Based on predicted environmental concentrations of carbaryl from both agricultural use and non-agricultural uses within the species' habitat of low flow/low volume habitats, we expect there will be a low likelihood of direct effects, including mortality (up to 0.17 % of individuals likely to die) and a low level of indirect effects through the loss of prey resources. We anticipate this level of direct and indirect effects will result in a low level of adverse effects to a large number of individuals. Therefore, we determine the overall risk of adverse effects to the species is low.

Conclusion

The Neuse River waterdog is a fully aquatic salamander that utilizes low to moderate-gradient streams with low current velocities but prefers riffles, runs, and pools in medium to large streams and rivers with moderate gradient. The species requires uncontaminated sites and is intolerant of degraded water quality as from siltation or turbidity so that, in general, stream channels with forested and stable banks where erosion is limited are more likely to support the species than sites where vegetation and stream banks have been altered (i.e., where agriculture or development activities exist). The Neuse River waterdog has a high vulnerability based on its status, distribution, and trends. Because the species is aquatic, we expect exposure to occur through spray drift and runoff. The agricultural labeled uses across the range are estimated to be high at 34.7% and agricultural usage is high with up to 11.3% of the ranged treated annually with carbaryl. The species' range includes some non-agricultural use sites, including developed areas and rights of way, but we do anticipate non-agricultural uses will result in the exposure of no more than a small number of individuals. In the high flow/volume waterbodies where the Neuse waterdog is primarily found, we do not expect mortality of exposed individuals. Very few individuals in low flow or low volume waterbodies may die from exposure. There is some potential for reduced fecundity and offspring survival in low flow or low volume waterbodies, but the Neuse River water dog prefers waterbodies with moderate currents for breeding and nesting. Additionally, where localized effects (e.g., reductions in prey) occur as a result of applications of carbaryl, we anticipate additional food resources from upstream sources will quickly recolonize, or individuals will seek out other areas of available prey. The species may experience a low level of temporary indirect effects from invertebrate prey loss.

Thus, we anticipate a large number of individuals of this species and their prey will experience exposure over the duration of the action. We expect low levels of mortality and low levels of reduced fecundity and offspring survival for exposed individuals. We anticipate the loss of or reduced reproduction in a small number of individuals will not result in species-level effects. After adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the survival and recovery of the species in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of the Neuse River waterdog.

References

- U.S. Fish and Wildlife Service. 2023. Draft Recovery Plan for the Neuse River Waterdog (*Necturus lewisi*). Raleigh, North Carolina. 17 pp.
- U.S. Fish and Wildlife Service. 2021a. Species Status Assessment Report for the Neuse River Waterdog (*Necturus lewisi*). Version 1.2. February 2021. Atlanta, Georgia.

Appendix C-A1. Amphibians: Integration and Synthesis Summaries

U.S. Fish and Wildlife Service. 2021b. Endangered and Threatened Wildlife and Plants; Threatened Species Status With Section 4(d) Rule for Neuse River Waterdog, Endangered Species Status for Carolina Madtom, and Designations of Critical Habitat. Final Rule. Federal Register 86(109): 30688-30751.

Integration and Synthesis Summary: Reticulated flatwoods salamander

Scientific Name:	Common Name:	Entity ID:
<i>Ambystoma bishopi</i>	Reticulated flatwoods salamander	9943

Species Overview

In reviewing the status of the species, species' range (Figure 3), and the environmental baseline and cumulative effects for the action area, the Service has determined that the species' vulnerability is high. In our evaluation of the effects of the proposed action to the species, we determine there is high overlap of the action area with the species' range and high past usage of carbaryl within the species' range. However, these data do not reflect a recent (2022) species' range update. After accounting for the expected overestimation in the overlap and usage calculations, we determine the species has a low extent of exposure. Though we do not expect adults will die from terrestrial dietary exposure, there may be low levels of mortality and sublethal effects in low flow/volume waterbodies where the species breeds. We expect reductions in some prey items from carbaryl, and because the species is a prey generalist, we expect temporary and low indirect effects from loss of prey. As such, we determine the risk of adverse effects to the species is medium. Even though we expect a small number of individuals are likely to experience indirect adverse effects from the proposed action, we do not expect species-level effects will occur. After adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the survival and recovery of the species in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of the reticulated flatwoods salamander. We discuss our rationale for this conclusion for the species in the sections below.

Species range

Based on range map dated: 1/28/2022; Wherever found; *States within the range:* FL, GA

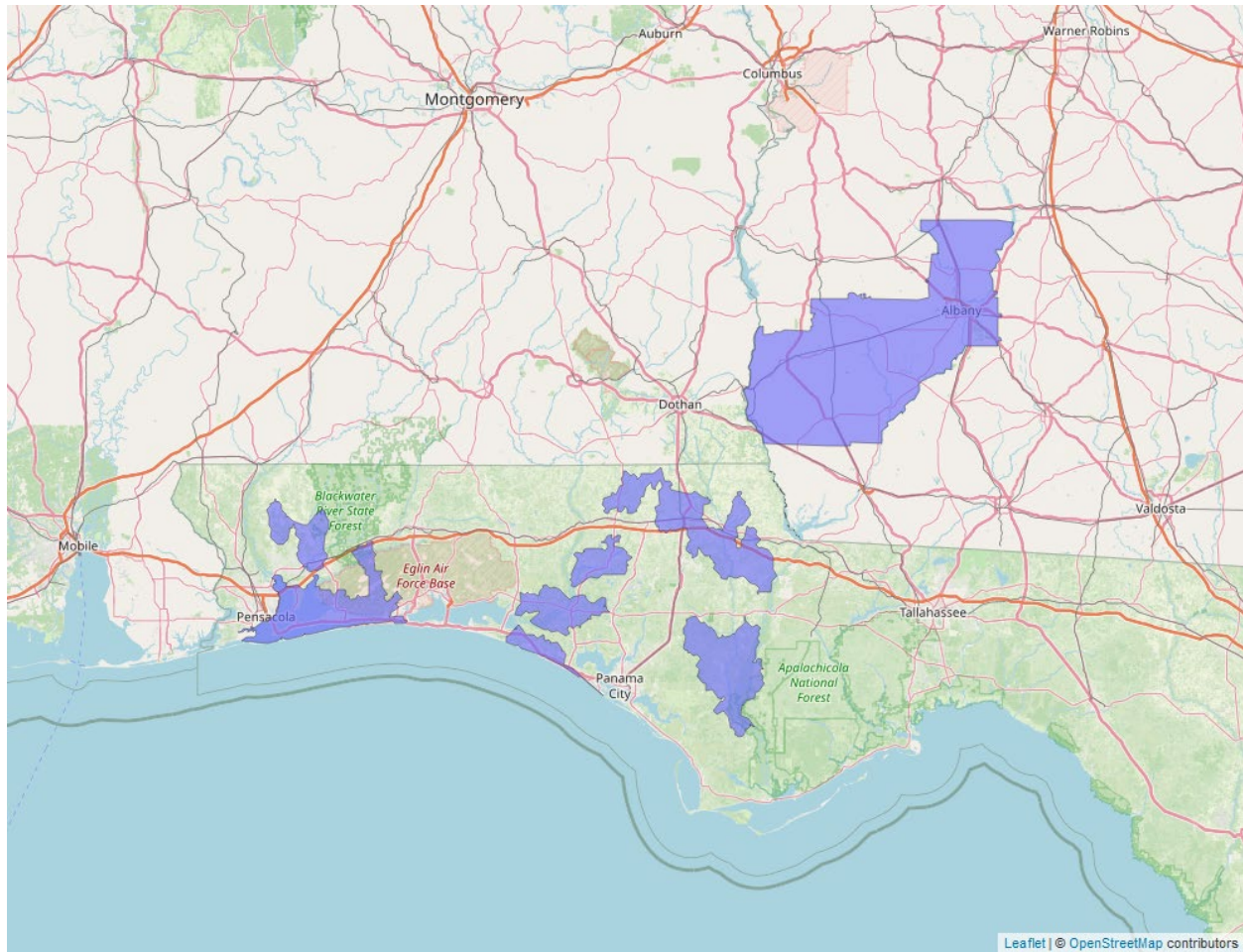


Figure 3. Range map of reticulated flatwoods salamander (blue polygons). Range map accessed at <https://ecos.fws.gov/ecp/species/8939>.

Vulnerability

Vulnerability of the species considers the status of the species, environmental baseline, and cumulative effects, as summarized below.

Summary of status

Listing status: Endangered

Most recent 5 Year Status Review recommendation: No change in Status

Most recently completed 5 Year Status Review: 5/25/2023

Distribution: Small, endemic, constrained, and/or isolated population(s)

Number of populations: Multiple populations (few)

Species trends: Declining population(s) - one or more populations declining

Pesticides noted in Service documents as a threat to the species: yes

Environmental Baseline/Cumulative Effects (EB/CE) Summary

The reticulated flatwoods salamander is an amphibian with a complex life cycle (i.e., aquatic larval stage, terrestrial egg and metamorphosed juvenile and adult stages) that breeds in ephemeral wetlands. Flatwoods salamander adults migrate to ephemeral (seasonally flooded) wetlands to breed in the fall. Juveniles usually disperse from ponds shortly after metamorphosing but may remain nearby during drought periods. Juveniles and adults are highly fossorial, spending much of their time in crayfish burrows or root channels until they reach sexual maturity at 1-2 years old. The reticulated flatwoods salamander was historically found in four southern counties of Alabama, but it has not been observed there since 1981. In Georgia, the reticulated flatwoods salamander was discovered in two wetlands on the Mayhaw Wildlife Management Area in Miller County. In Florida, the reticulated flatwoods salamander has been observed in Santa Rosa and Okaloosa Counties (17 breeding wetlands and four larvae detections). At the end of the 2014/2015 breeding season, there were six known and currently occupied populations across these wetlands in Florida and Georgia (USFWS 2020).

The main threat to the reticulated flatwoods salamander is loss of both its longleaf pine/slash pine flatwoods terrestrial habitat and its isolated, seasonally inundated breeding habitat. The combined pine flatwoods (longleaf pine-wiregrass and slash pine flatwoods) historical acreage was approximately 32 million acres. Flatwoods acreage was reduced to 5.6 million ac or approximately 18% of its original extent by conversions to urban development and agriculture. Remaining pine flatwoods (non-plantation forests) are typically fragmented and degraded by roads and pine plantations, with second-growth forests resulting from fire suppression. Most flatwoods salamander populations are widely separated from each other by unsuitable habitat. Flatwoods salamander breeding sites have been degraded or altered through alterations in hydrology, agricultural and urban development, road construction, incompatible silvicultural practices, shrub encroachment, dumping in or filling of ponds, conversion of wetlands to fish ponds, domestic animal grazing, and soil disturbance. Nonindigenous feral swine can significantly impact flatwoods salamander breeding sites through rooting. Invasive plant species such as cogongrass (*Imperata cylindrica*) threaten to further degrade existing habitat. Direct threats to flatwoods salamanders include disease and predation (i.e., fish and red imported fire ants [*Solenopsis invicta*]). Disease is currently unknown in natural populations of flatwoods salamanders, though a parasitic nematode (*Hedruris siredonis*) was found in South Carolina and Florida in larval flatwoods salamanders, and they may be susceptible to ranaviruses and chytrid fungus. Exposure to increased predation by fish is a potential threat to flatwoods salamanders when isolated, seasonally ponded wetland breeding sites are changed to, or connected to, more permanent wetlands inhabited by fishes that are not typically found in temporary wetlands.

Climate change, especially in combination with other stressors, is a daunting challenge for the persistence of amphibians. Sea level rise is becoming and will likely continue to increase as a threat to the extant populations of the frosted flatwoods salamanders. Most of the remaining populations occur in very low-lying areas within a short distance of the coast. Small population sizes, especially concentrated in small areas, are more susceptible to stochastic events that could negatively impact the entire population. Hurricane Michael in 2018 inundated many flatwood salamander ponds with salt water and the 2019 breeding season was believed to be near complete failure at St. Marks. Pesticides and herbicides may pose a threat to amphibians such as the flatwoods salamanders because their permeable eggs and skin readily absorb substances from the surrounding aquatic or terrestrial environment (USFWS 2015, 2020, 2023).

Overall Vulnerability: High

Effects of the Action: Exposure

Overlap

Data indicate that 29.5% of the species' range overlaps with agricultural use sites and 21.9% of the species' range overlaps with areas adjacent to use sites that are likely exposed through off-site transport (e.g., through spray drift or runoff). In total, there is approximately 51.4% overlap between the species' range and the agricultural footprint of carbaryl use (Table 9).

Usage

Past usage data indicate that up to 18% of the species' range has been treated with carbaryl annually from agricultural uses.

Table 9. Overlap of carbaryl use sites with reticulated flatwoods salamander.

Use Layer	Use Site Overlap (% range)	Off-Site Overlap (% range)	Total Overlap (% range)	% Range Treated On-Site	% Range Treated Off-Site	% Total Range Treated
Alfalfa	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Citrus	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Corn	7.4	4.9	12.3	1.2	1	2.1
Grapes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Other Crops	3.2	4.8	7.9	3.2	4.8	7.9
Other Grains	1.2	2.1	3.4	0.1	0.2	0.3

Use Layer	Use Site Overlap (% range)	Off-Site Overlap (% range)	Total Overlap (% range)	% Range Treated On-Site	% Range Treated Off-Site	% Total Range Treated
Other Orchards	2.4	2.7	5.1	1.2	1.4	2.7
Other Row Crops	14.9	7	21.9	2.3	1.1	3.4
Soybeans	1.3	1.9	3.2	1.3	1.8	3.2
Vegetables and Ground Fruit	0.4	0.4	0.8	0.3	0.2	0.5
Total	29.5	21.9	51.4	8.4	9.6	18

Additional Exposure Considerations

As adults, flatwoods salamanders migrate to ephemeral (i.e., seasonally flooded) wetlands to breed in the fall, where females lay eggs singly or in small clusters on litter, vegetation, or soil, usually in small depressions near the base of plants, in dry areas that will later fill with water provided by winter rainfall. Well-developed embryos hatch into larvae in the winter and metamorphose between March and May after an 11- to 18-week larval period. Juveniles normally disperse from wetlands shortly after metamorphosing but may stay near wetlands during seasonal droughts. Juveniles and adults are highly fossorial and spend much of their time in crayfish burrows or root channels until they reach sexual maturity (1 year for males; 1-2 years for females) and most return to their natal wetland to breed during the fall months (USFWS 2020).

The Service revised the species' range map in February of 2022 (after the submittal of the final BE), removing many areas that may historically have been habitat, but are no longer capable of supporting the species due to land use changes. Thus, we anticipate the use and usage information significantly overestimate overlap. While the species' habitat (a mosaic of pine dominated flatwoods and seasonal wetlands) sometimes exists adjacent to agricultural sites, it is not anticipated to overlap them.

Non-agricultural Uses

The reticulated flatwoods salamander occurs in ephemeral (seasonally flooded) wetlands and pine flatwoods, including some where forest harvest occurs. Available data on past carbaryl usage in managed forests from the U.S. Forest Service from 2016-2020 indicate no carbaryl has been used by the Forest Service within the range of the species. Where applications have taken place, the majority of treatments have involved small areas (<1 acre). As such, we anticipate a low likelihood of carbaryl usage in the range, and that if usage did occur, exposure to the reticulated flatwoods salamander would be minimal. In addition, rights of way, roadside ditches,

and borrow pits may be used as suboptimal habitat, especially if they are located near natural breeding wetlands. Available usage data indicate very little carbaryl usage is likely to occur in rights of way, with less than 500 pounds of carbaryl applied to roadways nationally each year. While this may result in a large treatment footprint if all rights of way usage were concentrated in one location or within the salamander's range, we expect this is highly unlikely to occur and rather expect rights of way usage is likely to be sporadic across the national landscape, with only small amounts, if any, used within the species' range. As such, we do not expect non-agricultural uses will result in the exposure of more than a small number of individuals.

Exposure Summary

Given the species' habitat preferences and revised range mapping that removed areas of historical habitat, we anticipate there is a low extent of overlap between agricultural use sites and the species' range, and as such, a low extent of usage for agriculture. Given that the extent of overlap is low, and that expected usage is low we expect a small number of individuals are likely to experience exposure from the proposed action.

Individuals may occur near non-agricultural use sites, including managed forests and rights of way areas. However, based on the low likelihood of usage within these non-agricultural uses, we do not anticipate exposure from non-agricultural uses will result in the exposure of more than a small number of individuals.

Overall Exposure Ranking: Low

Conservation Measures

Rain restriction: The carbaryl label has language designed to reduce the likelihood of pesticide runoff from use sites. This rain restriction language provides for a reduction in the concentration of carbaryl in aquatic habitats by providing time for carbaryl to degrade before runoff into aquatic habitats can occur, decreasing exposure and risk to the Salado salamander directly. Indirect effects to arthropod dietary items remain high for this species.

Aquatic habitat buffers: Application buffers are designed to reduce spray drift from entering sensitive non-target areas, thereby providing protection to aquatic species. While the exact amount of spray drift reduction depends on the physical traits of the aquatic ecosystem (e.g. flow rate, volume, etc.) as well as the application method, we can expect (based on AgDRIFT modeling) spray drift reductions with the spray drift mitigation practices already in place on the label. The carbaryl label has language to reduce the likelihood of pesticide spray drift from use sites specifically to nearby aquatic habitats. The label language states "Do not apply by ground equipment within 25 feet, or by air within 100 feet of lakes, reservoirs, rivers, estuaries, commercial fish ponds and natural, permanent streams, marshes or natural, permanent ponds."

We anticipate that, in many cases, these buffers will significantly reduce exposure to the reticulated flatwoods salamander and subsequent risk of direct effects and indirect effects to prey items.

Effects of the Action: Toxicity

Direct Effects

Because of its complex life cycle, the diet of the reticulated flatwoods salamander consists of aquatic prey consumed by larvae as well as terrestrial prey consumed by adults and juveniles.

We do not expect the reticulated flatwoods salamander will experience direct adverse effects from terrestrial dietary exposure. On-field exposure can result in dosages up to 22 mg/kg-bw, which can occur when individuals exclusively consume soil invertebrates on fields with crops using the highest application rate (5 lbs/acre). This level of exposure on-field will not cause mortality or other adverse effects to exposed individuals. In addition, because the reticulated flatwoods salamander has specific habitat requirements for feeding, breeding, and sheltering (mostly fossorial) and do not travel far from these areas to forage, it is unlikely they will forage on-field. They may forage near agricultural areas as their habitat is surrounded by large tracts of agricultural land, but we do not expect them to die from this exposure. We expect dietary dosages from consuming contaminated food items off-field will result in even lower levels of exposure to carbaryl.

Aquatic phase:

EPA's aquatic exposure modeling indicates that habitats within the regions that the reticulated flatwoods salamander occupies will likely be exposed to carbaryl at concentrations up to 873 µg/L, depending on the type of habitat and region. Based on this exposure, we expect, on average, 0.18% of exposed individuals will die in low flow/low volume waterbodies (wetlands) where their habitat is found. We anticipate a reduction in fecundity and offspring survival in some low flow/low volume waterbodies.

Indirect Effects

Based on available life history information, we expect the reticulated flatwoods salamander larvae rely on freshwater crustaceans as their main dietary item. Whiles (2004) documented that freshwater crustaceans comprise 96% of all invertebrates consumed by larval reticulated flatwoods salamanders. We expect some reductions in freshwater crustaceans (isopods and amphipods) from carbaryl exposure. However, based on carbaryl's low persistence in water and planktonic drift, we anticipate any localized reductions in zooplankton as a food source will be temporary and quickly replenished by upstream sources. As such, we do not anticipate any indirect adverse effects are likely to occur for larvae or metamorph dietary items.

Adult and juvenile reticulated flatwoods salamanders while spending most of their time in crayfish burrows within intermediate moisture-pine dominated flatwoods/savanna communities, feed on soil invertebrates, which are likely to experience adverse effects from carbaryl exposure. The reticulated flatwoods salamander will also feed on other amphibians and invertebrate species as well, therefore only low levels of indirect effects to their food base overall are anticipated as they have a variety of dietary items on which to forage.

Toxicity Summary

We do not expect adverse effects from terrestrial exposure. For larvae, we expect low mortality based on their feeding and presence in low flow aquatic habitats and we expect sublethal effects to reproduction are likely to occur at predicted exposure levels. We expect a moderate level of indirect effects are likely to occur to individuals as we anticipate carbaryl exposure will kill some aquatic isopods and amphipods that make up the diet for larval reticulated flatwoods salamanders, but these reductions will be temporary and prey items will be replenished soon after from upstream sources. For adults and juveniles that feed on soil invertebrates as well as other terrestrial dietary items we anticipate some reductions in soil invertebrates. However, this will not impact the salamander overall as they have a variety of dietary items on which to forage. Overall, we determine the reticulated flatwoods salamander has a medium toxicity ranking.

Overall Toxicity Ranking: Medium

Effects of the Action Summary

The reticulated flatwoods salamander has a low exposure ranking. Based on an assessment of past carbaryl usage data, we expect up to 18% of the range may be treated annually but may potentially cover up to 51.4% of the range over the duration of the proposed action depending how usage patterns may or may not change over time. However, the Service revised the species range map in February of 2022, removing many areas that may have historically included habitat, but no longer are capable of supporting the species. Thus, we anticipate the agricultural use and usage data represent significant overestimates given the species habitat preferences of a mosaic of pine dominated flatwoods and seasonal wetlands. We anticipate only a small portion of the species' range is likely to be treated overall for agricultural purposes. As such, we expect a small number of individuals are likely to be exposed to carbaryl. Individuals may occur near non-agricultural use sites, including managed forests and rights of way. However, based on the species' habitat preferences for pine flatwoods, low levels of past usage, and existing mitigation measures on product labels, we do not anticipate non-agricultural uses will result in the exposure of more than a small number of individuals.

The reticulated flatwoods salamander has a medium toxicity ranking. We do not anticipate adverse effects from dietary exposure through the consumption of contaminated food items to adults and juveniles during the terrestrial phase of the life cycle on or off-field. We expect a low level of indirect adverse effects are likely to occur as we expect prey species in the aquatic

waterbodies where the larvae feed will experience some mortality with exposure to predicted concentrations of carbaryl however, this will not reduce the prey items for the reticulated flatwoods salamander larvae extensively as these prey items can be replenished in a short amount of time from upstream sources. We also anticipate a reduction in fecundity and offspring survival in low flow/low volume habitats.

We expect a small number of individuals are likely to be exposed from agricultural uses. In addition, we expect a low level of indirect adverse effects and a reduction in fecundity and offspring survival for individuals exposed. Thus, we determine the overall risk of adverse effects to the species is medium.

Conclusion

The reticulated flatwoods salamander has a high vulnerability ranking due to its threatened status (with a 5-year review recommendation to uplist to endangered), limited distribution, small population size, low juvenile survival rates, susceptibility to stochastic events, and anthropogenic threats to the species (e.g., climate change, continued degradation, fragmentation and loss of suitable aquatic and upland habitats from urbanization, invasive species, fire suppression, and agricultural impacts to habitat). The species has a low exposure ranking because we anticipate the species' habitat preferences will keep it off-field and limit exposure within the revised species' range. While labeled uses across the range estimated agricultural carbaryl usage affecting 18% of the species range annually and up to 51.4% of the former species' range overlaps carbaryl use sites, we anticipate these estimates are significant overestimates. Effects to prey items on use sites and exposure of reticulated flatwoods salamanders from ingestion of contaminated soil-based prey are anticipated to be rare events. Based on the reclusive behavior and specialized habitat preferences of the species (i.e., fossorial lifestyle), we anticipate foraging (including exposed soil-based invertebrate prey), seasonal breeding, and dispersal activity for terrestrial life stages of the species will expose only small numbers of individual salamanders and their prey over the duration of the proposed action. The species' range includes some non-agricultural use sites, including managed forests and rights of way, but we expect non-agricultural uses to result in the exposure of no more than a small number of individuals because non-agricultural carbaryl usage is expected to be low and use sites do not serve as preferred habitat for the salamander.

Amphibians in general are at high risk, given their aquatic life histories and susceptibility to environmental contaminants (e.g., pesticides, degraded water quality). They can be exposed through multiple pathways (e.g., dermal exposure, ingestion of contaminated arthropod prey) and at various life stages (e.g., egg, larval, juvenile, and adult). We do not expect mortality from terrestrial dietary exposure. We expect low levels of mortality, reductions in fecundity, and lower offspring survival in low flow/volume waterbodies where the species is found and exposed to carbaryl. We expect high levels of indirect effects from loss of prey, primarily crustaceans eaten by larval salamanders and some temporary loss of terrestrial prey species. Where localized effects (e.g., reductions in prey) occur as a result of applications of carbaryl, we anticipate

additional food resources from upstream sources will quickly recolonize, or individuals will seek out other areas of available prey.

Thus, we anticipate a low number of individuals of this species will experience moderate adverse effects over the duration of the action, primarily from loss of prey but also from exposure that is likely to result in mortality or effects to reproduction in a small number of individuals. We anticipate this moderate level of adverse effects will not result in species-level effects and the action is not expected to appreciably reduce the survival and recovery of the reticulated flatwoods salamander in the wild. Therefore, it is our biological opinion that the registration of carbaryl, as proposed, is not likely to jeopardize the continued existence of the reticulated flatwoods salamander.

References

U.S. Fish and Wildlife Service. 2023. Reticulated flatwoods salamander (*Ambystoma bishopi*), 5-Year Review: Summary and Evaluation. Panama City, Florida. 10 pp.

U.S. Fish and Wildlife Service. 2020. Species Status Assessment for the Reticulated flatwoods salamander (*Ambystoma bishopi*) Version 1.0. Panama City, Florida. 97 pp.

U.S. Fish and Wildlife Service. 2015. Reticulated flatwoods salamander (*Ambystoma bishopi*), 5-Year Review: Summary and Evaluation. Panama City, Florida.

Integration and Synthesis Summary: Dixie Valley toad

Scientific Name:	Common Name:	Entity ID:
<i>Anaxyrus williamsi</i>	Dixie Valley toad	11468

Species Overview

In reviewing the status of the species, species' range (Figure 4), and the environmental baseline and cumulative effects for the action area, the Service has determined that the species' vulnerability is high. In our evaluation of the effects of the proposed action to the species, we determine there is moderate overlap of the action area with the species' range and low past usage of carbaryl within the species' range. However, the species primarily occurs on federal lands (Department of Defense and Bureau of Land Management) where we do not expect carbaryl will be used, indicating a low extent of exposure. Though we do not expect exposed adults will die, the few that breed in low flow/volume waterbodies may experience reduced fecundity and offspring survival. We expect reductions in some prey items from carbaryl, and because the species is a prey generalist, we expect temporary and low indirect effects from loss of prey. As such, we determine the risk of adverse effects to the species is low. Even though we expect a small number of individuals are likely to experience indirect adverse effects from the proposed action, we do not expect species-level effects will occur. After adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the survival and recovery of the species in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of the Dixie Valley toad. We discuss our rationale for this conclusion for the species in the sections below.

Species range

Based on range map dated: 4/28/2022; *States within the range:* NV

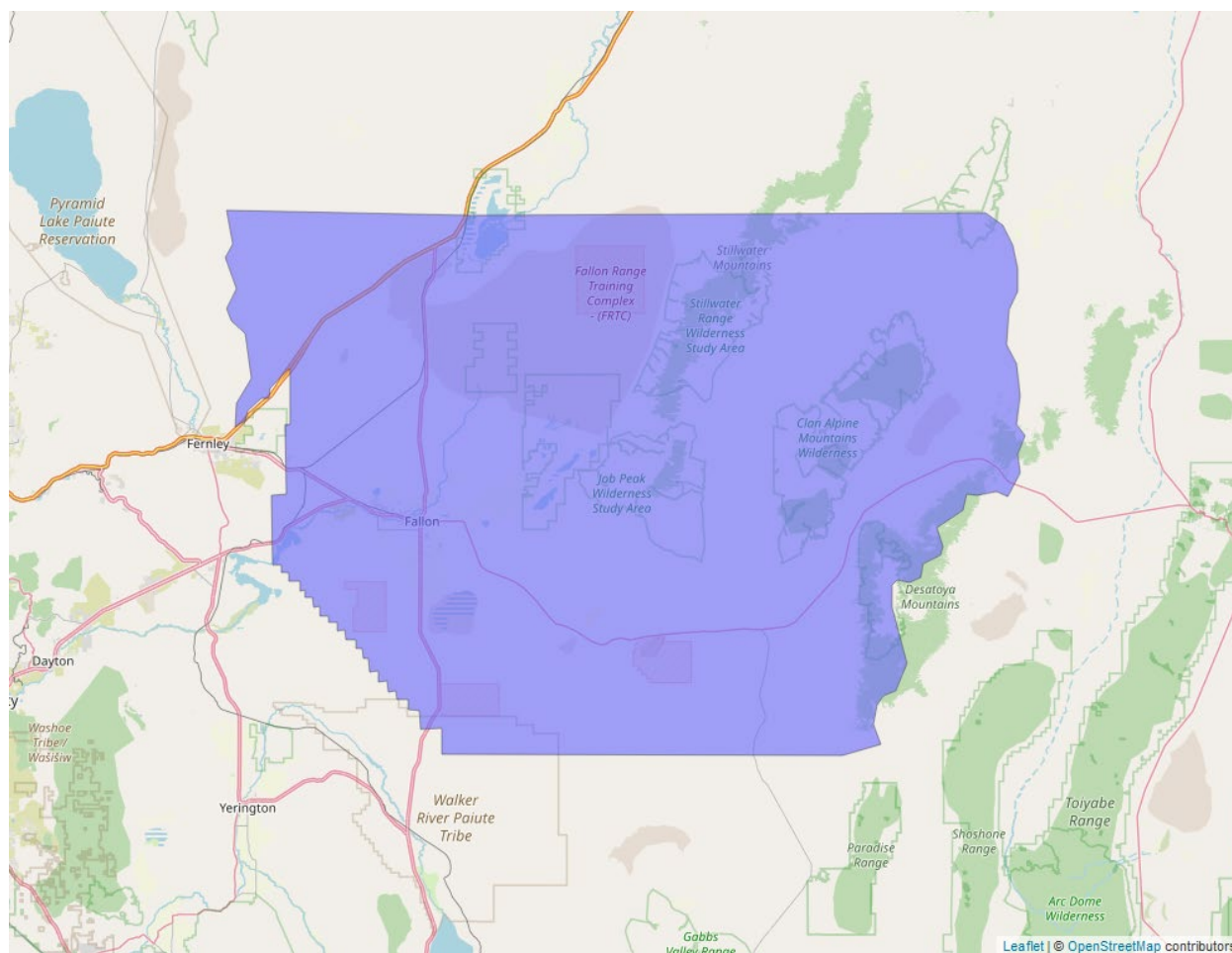


Figure 4. Range map of Dixie Valley toad (blue polygons). Range map accessed at <https://ecos.fws.gov/ecp/species/10635>.

Vulnerability

As mentioned above, vulnerability considers the present condition of the species to determine its vulnerability to additional stressors. Here, in making our jeopardy determination, vulnerability of the species is a function not only of its status, but also the environmental baseline and cumulative effects, as summarized below.

Summary of status

Listing status: Endangered

Most recent 5-Year Status Review recommendation: N/A

Most recently completed 5 Year Status Review: N/A

Distribution: Small, endemic, constrained, and/or isolated population(s)

Number of populations: Single population

Species trends: Declining population(s) - one or more populations declining

Pesticides noted in Service documents as a threat to the species: No

Environmental Baseline/Cumulative Effects (EB/CE) Summary

Dixie Valley toads are a narrow endemic toad found in a single metapopulation at Dixie Meadows, approximately 69 km northeast of the City of Fallon in Nevada. Dixie Meadows consists of six wetlands connected by upland habitat. The numerous springs and spring provinces in the Dixie Meadows discharge area represent a unique feature in Dixie Valley. Outside of the Dixie Meadows wetland, the surrounding landscape is characterized by expansive xeric habitats nearly devoid of surface water. Surface water flowing from Dixie Meadows springs are formed from a combination of shallow basin-fill aquifer, mainly recharged from atmospheric contributions which fall on the Stillwater Range, and a deep geothermal reservoir. Toads are rarely found farther than 14 m from aquatic habitats. They require sufficient wetted areas, adequate water temperature, wetland vegetation, and adequate water quality. Due to lack of specific information, we assume they are opportunistic feeders like other toads, primarily eating aquatic and terrestrial invertebrates as adults and algae and detritus as aquatic larvae. Dixie Meadows is managed by federal entities (i.e., Department of Defense and Bureau of Land Management), including all areas occupied by the Dixie Valley toad. Population estimates are unavailable for Dixie Valley toads, but consistent reproduction has been documented (USFWS 2023).

Threats to the species include geothermal development (i.e., changes in water temperature and flow, habitat loss), predation, disease, livestock grazing, spring modifications, groundwater pumping, and altered precipitation and temperature from climate change. Negative impacts are expected to occur to toads and their habitats from geothermal development, but the extent of these impacts is unknown. Heavy livestock grazing has been shown to negatively influence amphibian populations and their habitat. Dixie Meadows is grazed by livestock, but there is no indication of habitat loss due to the effects of heavy grazing. Spring modifications may include surface water diversion, impoundment, or channel modification, including dredging. These spring modifications affect Dixie Valley toad needs by changing how water is distributed throughout the wetland, and open water needed for plant productivity, which provides food and shelter. The most extreme effects of groundwater withdrawal on Dixie Valley toads are desiccation and extirpation or extinction. If groundwater withdrawal occurs but does not cause a spring to dry, there can still be adverse effects to Dixie Valley toads or their habitat (USFWS 2023).

Overall Vulnerability: High

Effects of the Action: Exposure

Overlap with Agricultural Use Sites

Data indicate that 3.9% of the species' range overlaps with agricultural use sites and 2% of the species' range overlaps with areas adjacent to use sites that are likely exposed through off-site transport (e.g., through spray drift or runoff). In total, there is approximately 5.9% overlap between the species' range and the agricultural footprint of carbaryl use (Table 10).

Usage

Past usage data indicate that up to 0.7% of the species' range has been treated with carbaryl annually from agricultural uses.

Table 10. Overlap of carbaryl use sites with Dixie Valley toad.

Use Layer	Use Site Overlap (% range)	Off-Site Overlap (% range)	Total Overlap (% range)	% Range Treated On-Site	% Range Treated Off-Site	% Total Range Treated
Alfalfa	2.3	0.6	2.9	0.5	0.1	0.6
Citrus	0	0	0	0	0	0
Corn	0.4	0.2	0.6	<0.1	<0.1	<0.1
Grapes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Other Crops	0.9	0.8	1.7	0	0	0
Other Grains	0.3	0.3	0.6	<0.1	<0.1	<0.1
Other Orchards	0	0	0	0	0	0
Other Row Crops	0	0	0	0	0	0
Soybeans	0	0	0	0	0	0
Vegetables and Ground Fruit	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total	3.9	2	5.9	0.6	0.2	0.7

Additional Exposure Considerations

Dixie Valley toads are endemic to Dixie Meadows, Churchill County, Nevada. Dixie Meadows is a ground water dependent ecosystem consisting of at least 122 springs and seeps located on the east side of the Stillwater Range. Approximately 90% of occupied habitat is located on Department of Defense lands and the remaining is on public lands managed by the Bureau of Land Management.

Non-agricultural Uses

The Dixie Valley toad habitat is very xeric in nature and located within Department of Defense and Bureau of Land Management lands. While there is likely very little carbaryl used for non-agricultural applications within these areas, there are residential areas outside of the range. We expect carbaryl use in these areas to have a low potential for off-site transport, as many applications in developed areas will be limited to hand-held equipment for spot and crack treatments (as discussed above in the exposure section of this document) that limits the amount of run-off that may enter nearby aquatic habitats where the Dixie Valley toad may be found. As such, we do not anticipate that these areas outside of the range will contribute to carbaryl exposure for the Dixie Valley toad. We do not expect carbaryl exposure to occur from non-agricultural uses.

Exposure Summary

There is a moderate extent of overlap between agricultural use sites and the species' range, but the species is only found on lands managed by the Department of Defense and Bureau of Land Management, where we expect agricultural pesticide use will be unlikely. Based on past agricultural usage data, we expect a low level of usage within the range. Therefore, we consider the extent of overlap and expected usage to be low, and we expect a small number of individuals are likely to be exposed from the proposed action.

Individuals may occur near non-agricultural use sites, including managed forests, developed, open space developed, and rights of way areas. However, based on the low likelihood of non-agricultural usage in the species habitat and conservation measures for limiting off-site transport of residential uses, we do not anticipate exposure from non-agricultural uses for the Dixie Valley toad.

Overall Exposure Ranking: Low

General Conservation Measures

Rain restriction: The carbaryl label has language designed to reduce the likelihood of pesticide runoff from use sites. This rain restriction language provides for a reduction in the concentration of carbaryl in aquatic habitats by providing time for carbaryl to degrade before runoff into

aquatic habitats can occur, decreasing exposure and risk to the Dixie Valley toad directly when in the aquatic phase as eggs or early metamorphs.

Aquatic habitat buffers: Application buffers are designed to reduce spray drift from entering sensitive non-target areas, thereby providing protection to aquatic species. While the exact amount of spray drift reduction depends on the physical traits of the aquatic ecosystem (e.g. flow rate, volume, etc.) as well as the application method, we can expect (based on AgDRIFT modeling) spray drift reductions with the spray drift mitigation practices already in place on the label. The carbaryl label has language to reduce the likelihood of pesticide spray drift from use sites specifically to nearby aquatic habitats. The label language states “Do not apply by ground equipment within 25 feet, or by air within 100 feet of lakes, reservoirs, rivers, estuaries, commercial fish ponds and natural, permanent streams, marshes or natural, permanent ponds.”

We anticipate that, in many cases, these buffers will significantly reduce exposure to the Dixie Valley toad and subsequent risk of direct effects and indirect effects to prey items.

Effects of the Action: Toxicity

Direct Effects

There is no published information on the feeding habits of Dixie Valley toads. It is assumed that adult Dixie Valley toads are opportunistic feeders, like other toad species (USFWS 2023), and their diet most likely consists of aquatic and terrestrial invertebrates found in Dixie Meadows. Aquatic larvae are assumed to feed on algae and detritus.

We do not expect the Dixie Valley toad to experience direct adverse effects, including mortality, from consuming contaminated terrestrial prey on treated sites or in adjacent areas exposed via off-site transport. Off-field exposure is not likely as the Dixie Valley toad is restricted to the wetted areas of the springs and seeps within its habitat and any areas outside of these habitats are not favorable to the Dixie Valley toad because they are dry and lacking surface water. Given that carbaryl use sites are not likely to occur within the species' range, we do not anticipate any individuals will be exposed to on-field levels of carbaryl.

Aquatic phase:

EPA's aquatic exposure modeling indicates that carbaryl can occur within the Dixie Valley toad's habitat at concentrations ranging up to 873 µg/L, depending on the type of habitat and region. We expect, at high end estimates, that up to 0.18% of exposed individuals are likely to die. This mortality would likely be limited to only tadpoles as juveniles and adults are semi aquatic and can avoid aquatic exposure by leaving contaminated waters. In contrast, at lower end estimates, we do not anticipate any exposed individuals are likely to die. At these estimated environmental concentrations, we also anticipate a reduction in fecundity and offspring survival in some low flow/low volume waterbodies.

Indirect Effects

Based on available life history information, we expect the Dixie Valley toad is an opportunistic forager that can consume plant matter (e.g., algae, plankton) during the tadpole phase and both terrestrial and aquatic invertebrates during the adult and juvenile phases. While we expect some reductions in the abundances of aquatic and terrestrial insects from carbaryl exposure, based on carbaryl's low persistence, we anticipate any reductions in sensitive aquatic prey species will be localized and dependent on the habitat type (e.g., low flow/low volume waterbodies will experience greater reductions of prey and longer recovery times as these habitats accumulate more carbaryl). Furthermore, given the breadth of dietary items individuals can use, we anticipate in situations where carbaryl use reduces the abundance of sensitive prey species, individual toads can switch to more abundant food resources. As such, we anticipate low levels of indirect adverse effects are likely to occur. We do not anticipate any indirect effects from dietary exposure during the tadpole phase as available toxicity data in aquatic plants indicate no reductions in plant survival or growth are likely to occur with carbaryl exposure.

Toxicity Summary

We do not expect direct adverse effects will occur because the species is not expected to occur on-field or near fields due to their habitat preferences. We expect low levels of mortality, reductions in fecundity and lower offspring survival in low flow/volume waterbodies where the species is found and exposed to carbaryl. We do not expect indirect effects from loss of prey because the species relies on diverse prey sources at different life stages. Overall, we determine the Dixie Valley toad has a low toxicity ranking.

Overall Toxicity Ranking: Low

Effects of the Action Summary

The Dixie Valley toad has a low exposure ranking. The species' range is entirely located on Department of Defense and Bureau of Land Management land. We do not anticipate any agriculture is likely occurring on these federal lands, nor do we anticipate non-agricultural uses of carbaryl will occur within the species' range. While individuals may be exposed to carbaryl residues from spray drift or runoff from nearby areas, we anticipate only a small number of individuals, at most, will be exposed to carbaryl. The Dixie Valley toad has a low toxicity ranking. While we do not anticipate effects to juvenile or adult toads, tadpoles occupying low flow or low volume waterbodies are likely to be exposed to carbaryl, resulting in low levels of mortality. We expect reduced reproduction (reduction in fecundity and offspring survival) is likely to occur at predicted exposure levels.

While there is a low level of toxicity and the potential for adverse effects to reproduction associated with exposure, particularly for breeding adults and tadpoles, we expect very few individuals are likely to be exposed from agricultural or non-agricultural uses given the location

of the species' range on federal lands and very little dispersal capability for the Dixie Valley toad. As such, we expect the overall risk of adverse effects to the species is low.

Conclusion

The Dixie Valley toad has a high vulnerability ranking due to its endangered status, limited distribution, small population size, and anthropogenic threats to the species (e.g., geothermal development (i.e., changes in water temperature and flow, habitat loss), predation, disease, livestock grazing, spring modifications, groundwater pumping, and altered precipitation and temperature from climate change). Population estimates are not available and based on the data we have; it is difficult to infer temporal trends or population size. In addition to adult toads, surveys recorded eggs, tadpoles, and juveniles in all survey years, suggesting consistent reproduction is occurring. Dixie Valley toads are primarily a wetted area species that rely on springs and spring provinces in the Dixie Meadows discharge area of Dixie Valley. Outside of the Dixie Meadows wetland, the surrounding landscape is characterized by expansive xeric habitats nearly devoid of surface water. Dixie Valley toads are restricted to spring areas and because toads are rarely encountered more than 14 meters from aquatic habitat, we have high confidence they do not disperse far.

The agricultural labeled uses across the range are estimated to be moderate at 5.9% and agricultural usage is low with up to 0.7% of the ranged treated annually with carbaryl. Because the species is restricted to wetted areas in its habitat and we do not expect carbaryl use sites occur within the species' occupied range, we do not expect Dixie Valley toads to occur on-field. We anticipate the likelihood of exposure to carbaryl is low, stemming mostly from the presence of their very specialized habitat on Department of Defense and Bureau of Land Management lands where very little use of carbaryl is likely. The species' range includes some non-agricultural use sites, including managed forests, developed, open space developed, and rights of way, but we do not expect those routes of exposure to occur based on low past usage and conservation measures in place for residential uses that limit off-site transport of carbaryl.

We do not expect direct adverse effects will occur through terrestrial dietary exposure. We expect low levels of mortality, reductions in fecundity, and lower offspring survival in low flow/volume waterbodies where the species is found and exposed to carbaryl. We expect low levels of indirect effects from loss of prey because the species relies on diverse prey sources at different life stages, and some may die from carbaryl exposure. While there are likely to be some reductions of available invertebrate prey adjacent to agricultural use sites, we do not anticipate this will impact the species as a whole because they are algae feeders during the larval and juvenile metamorph phases. Any aquatic invertebrate prey they consume will decline in abundance in low flow or low volume waters and will be replenished quickly over time.

We anticipate exposure to aquatic phases (i.e., egg and larval life stages) from runoff and spray drift and mortality of individuals at natal ponds across portions of the range where reproductive sites exist adjacent to agriculture use sites. Vulnerability of the aquatic life stage is high, and we anticipate toxic concentrations of carbaryl in the aquatic environment, particularly in smaller, low flowing habitats where tadpoles and early metamorphs are found that will result in low

Appendix C-A1. Amphibians: Integration and Synthesis Summaries

levels of mortality. We anticipate the 48-hour rain restriction measure and aquatic habitat buffers on the label will be sufficient to protect the Dixie Valley toad throughout its lifecycle.

Thus, we anticipate small numbers of individuals of this species will die or experience adverse effects to reproduction from direct exposure and prey losses over the duration of the action. We anticipate the loss of a small number of individuals will not result in species-level effects and the action is not expected to appreciably reduce the survival and recovery of the species in the wild. Therefore, it is our biological opinion that the registration of carbaryl, as proposed, is not likely to jeopardize the continued existence of the Dixie Valley toad.

References

U.S. Fish and Wildlife Service. 2023. Species Status Assessment for Dixie Valley Toad (*Anaxyrus williamsi*) Churchill County, Nevada. Version 1.2. Reno, Nevada. 131 pp.

Integration and Synthesis Summary: Llanero coquí

Scientific Name:	Common Name:	Entity ID:
<i>Eleutherodactylus juanariveroi</i>	Llanero coquí	9378

Species Overview

In reviewing the status of the species, the environmental baseline, and cumulative effects for the action area, we determined that the species' vulnerability is high. In our evaluation of the effects of the proposed action to the species, we determined there is a low extent of exposure. A moderate portion of the range overlaps with agricultural areas, and a very small portion of the range overlaps with areas subject to spray drift from agricultural areas. We expect an even smaller portion of the range has been exposed to insecticides in the past based on Census of Agriculture data for Puerto Rico. We do not expect the species to occur or forage on-field, and we do not expect mortality off-field from dietary exposure. Some insect prey species may die from carbaryl exposure on-field and off-field, but we do not expect more than low levels of indirect effects to coquíes from loss of prey. We determined the risk of adverse effects to the species is low. As such, we expect only a small number of individuals are likely to experience reduced feeding success from the proposed action. After adding the effects of the action and cumulative effects to the environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the survival and recovery of the species in the wild. Thus, it is our biological opinion that the proposed action is not likely to jeopardize the continued existence of the Llanero coquí. We discuss our rationale for the species in the sections below.

Species range

Based on range map dated: 08/20/2021; Wherever found; *States within the range:* PR

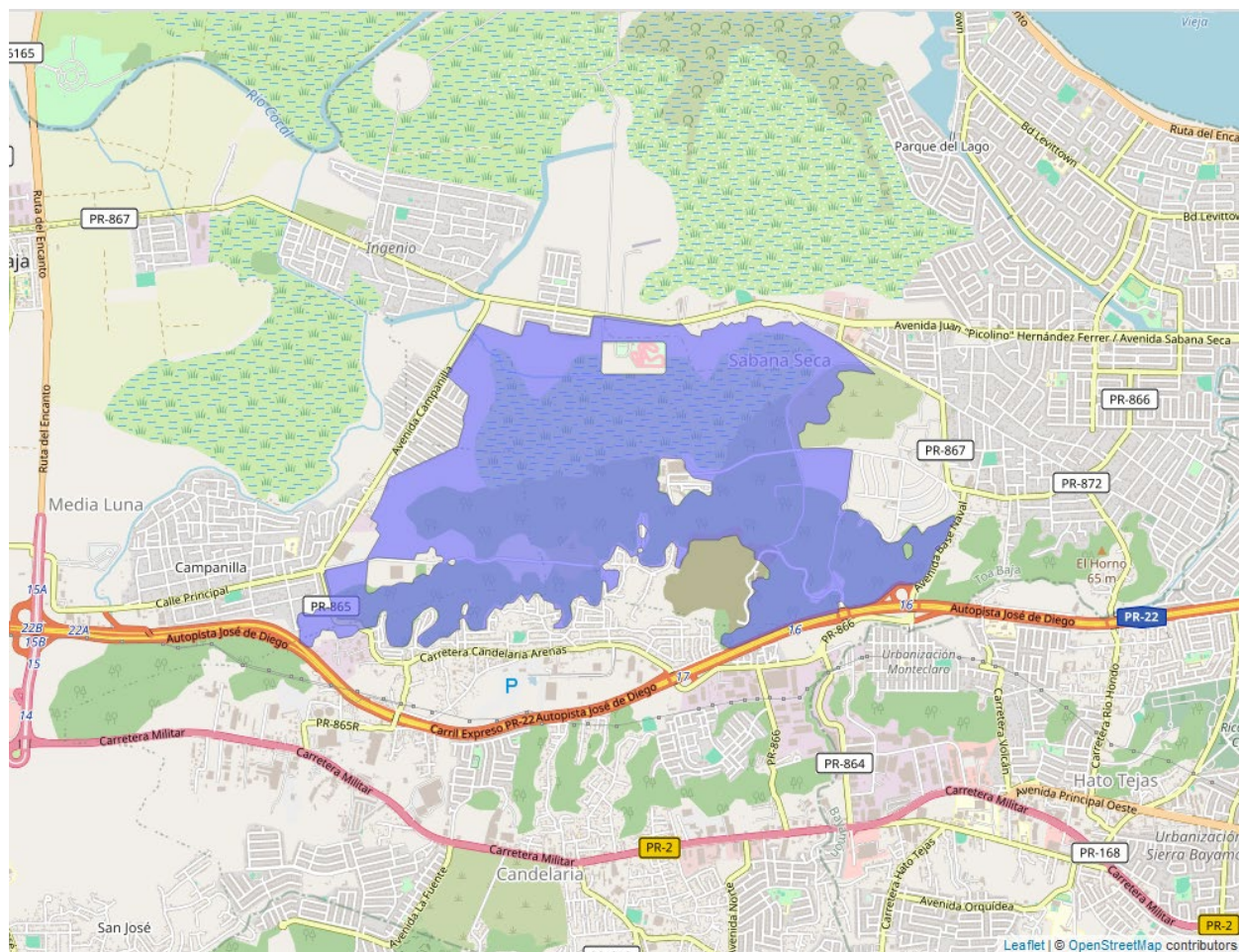


Figure 5. Range map of llanero coquí (blue polygons). Range map accessed at <https://ecos.fws.gov/ecp/species/D03V>.

Vulnerability

As mentioned above, vulnerability considers the present condition of the species to determine its vulnerability to additional stressors. Here, in making our jeopardy determination, vulnerability of the species is a function not only of its status, but also the environmental baseline and cumulative effects, as summarized below.

Summary of status

Listing status: Endangered

Most recent 5-Year Status Review recommendation: No change in Status

Most recently completed 5 Year Status Review: 6/17/2024

Distribution: Small, endemic, constrained, and/or isolated population(s)

Number of populations: Single population

Species trends: Unknown population trends

Pesticides noted in Service documents as a threat to the species: yes

Environmental Baseline/Cumulative Effects (EB/CE) Summary

The llanero coquí is the smallest coquí species in Puerto Rico, about the size of a dime when fully grown. By the time the previous 5-Year Review was published (August 2019; Service 2019), only one llanero coquí population was known in the Sabana Seca wetland area in Toa Baja with an estimated population of 473.3 ± 186.8 individuals per hectare or 192 per acre from surveys conducted in 2005-2006. Since then, two new populations have been described further west and east from the type locality in Sabana Seca. In 2018, a second breeding population of llanero coquí was found and confirmed in the Caño Tiburones area in Arecibo. This population is approximately 45 kilometers (30 miles) west from Sabana Seca. In 2023, a third breeding population was found and confirmed in Carolina, approximately 28 kilometers (17 miles) east from Sabana Seca. The extent of these two new populations is being investigated. Visits to other nearby suitable wetland locations further east yielded no records for the species but still warrant further exploration (USFWS 2024).

Due to the species restricted range, stochastic events such as fire are a major concern for this species. Additionally, contaminants, such as herbicide runoff and landfill leachate pollution, are a major concern that could impact the aquatic environment in which this species depends. The llanero coquí is highly restricted in its range and the threats occur throughout its range.

Overall Vulnerability: High

Effects of the Action: Exposure

Overlap with Agricultural Use Sites

We expect 5.5% of the species' range will overlap with carbaryl use sites or is likely to be exposed through off-site transport within the action area (Table 11). Up to 4.6% of the species' range occurs on carbaryl use sites while 0.96% of the range occurs off-field and may be exposed through spray drift and runoff.

Table 11. Overlap of carbaryl use sites with the llanero coquí range.

Use Layer	On-field Overlap (% range)	Off-field Overlap (% range)	Total Overlap (% range)
Cultivated land layer	4.6	0.96	5.5

Usage

Past carbaryl usage data in Puerto Rico is unavailable. However, Census of Agriculture data in Puerto Rico indicate that insecticide usage occurs on 20-70% of crops annually per municipality, with carbaryl presumably being among those insecticides. We broadly use this data as confirmation that carbaryl usage likely occurs within the species' range.

Additional Exposure Considerations

The llanero coquí is an herbaceous wetland specialist found only on a palustrine herbaceous wetland at Sabana Seca Ward previously managed by the U.S. Naval Security Group Activity Sabana Seca and areas owned by the Commonwealth of Puerto Rico (i.e., University of Puerto Rico and Puerto Rico Land Authority). The Service estimated the palustrine herbaceous wetland area where the llanero coquí is now found to be about 615 acres (249 hectares). The species appears to be an obligate marsh dweller and has been found only in freshwater, herbaceous wetland habitat at an elevation of 55.8 feet (17 meters).

The llanero coquí exhibits direct development by laying eggs outside of the water (such as other *Eleutherodactylus*) and does not have an aquatic, free swimming larval stage (tadpole) as most frogs do. The egg masses of the llanero coquí are enclosed on a thick jelly coat and placed on the plant *Sagittaria lancifolia* (bulltongue arrowhead) in leaf axils or leaf surfaces. Contrary to most species in the same genus, the llanero coquí does not provide parental care to the egg mass. The jelly coat is unique among Puerto Rican *Eleutherodactylus* species and is an important adaptation in the absence of parental care because it may protect eggs from dehydration, predation, and from microbial/fungi overgrow (USFWS 2019). Once eggs have developed, a tiny froglet hatches and has the same appearance as an adult.

The life history of other frogs in the genus *Eleutherodactylus* indicates they are opportunistic feeders where diets reflect the availability of food of appropriate size (USFWS 2019). The wetland appears to provide a variety of food sources for the species, mostly small insects and other invertebrates.

Non-agricultural Uses

Llanero coquí are not commonly found on non-agricultural areas and are not likely to disperse. They are strictly palustrine wetland species. However, the current known population locations are surrounded by open space developed, developed, and rights of way areas. We expect many carbaryl applications in developed areas will be limited to hand-held equipment for spot

treatments, methods that limit the amount of runoff that may enter nearby aquatic habitats where the llanero coquí may be found. We do not have non-agricultural usage data for Puerto Rico, so we cannot rule out exposure to carbaryl for the llanero coquí from non-agricultural carbaryl uses.

Exposure Summary

Individuals may occur near but not within agricultural or non-agricultural use sites, including developed, open space developed, and rights of way areas. Even though we expect limited occurrence in or adjacent to carbaryl use sites, exposure is likely low for some non-agricultural application methods (i.e., hand-held equipment), and existing mitigation measures on product labels will further limit exposure. However, we cannot rule out exposure for the llanero coquí from agricultural or non-agricultural carbaryl uses. We anticipate a low likelihood of exposure from both agricultural and non-agricultural uses of carbaryl, and we expect a small number of individuals are likely to experience exposure from the proposed action.

Overall Exposure Ranking: Low

Conservation Measures

Rain restriction: The carbaryl label has language designed to reduce the likelihood of pesticide runoff from use sites. This rain restriction language provides for a reduction in the concentration of carbaryl in aquatic habitats by providing time for carbaryl to degrade before runoff into aquatic habitats can occur, decreasing exposure and risk to the llanero coquí directly in their wetland habitat.

Aquatic habitat buffers: Application buffers are designed to reduce spray drift from entering sensitive non-target areas, thereby providing protection to aquatic species. While the exact amount of spray drift reduction depends on the physical traits of the aquatic ecosystem (e.g. flow rate, volume, etc.) as well as the application method, we can expect (based on AgDRIFT modeling) spray drift reductions with the spray drift mitigation practices already in place on the label. The carbaryl label has language to reduce the likelihood of pesticide spray drift from use sites specifically to nearby aquatic habitats. The label language states “Do not apply by ground equipment within 25 feet, or by air within 100 feet of lakes, reservoirs, rivers, estuaries, commercial fish ponds and natural, permanent streams, marshes or natural, permanent ponds.”

We anticipate that, in many cases, these buffers will significantly reduce exposure to the llanero coquí and subsequent indirect effects to prey items.

Effects of the Action: Toxicity

Direct Effects

We expect terrestrial phase amphibians will be directly exposed to carbaryl through dietary exposure. The llanero coquí primarily is an opportunistic feeder and consumes mostly insects and small arthropods. Because we know the llanero coquí is most likely to feed off-field, we do not anticipate mortality from feeding on invertebrates in their wetland habitat. The habitat for the llanero coquí is palustrine wetlands, and we do not anticipate EECs for this habitat will exceed 601.6 µg/L. We anticipate no mortality of the coquí and only minor impacts to reproduction (i.e., reduced fecundity) may occur because reproductive activity (mating, egg laying, etc.) takes place outside of water, on the leaf axils or leaf surfaces of *Sagittaria lancifolia* (bulltongue arrowhead).

Indirect Effects

Based on available toxicity data, we expect prey individuals will likely experience high levels of mortality with exposure to carbaryl, with greater mortality expected on-field than off-field. As such, we expect there may be substantial reductions in the abundance of invertebrate prey species where use sites abut preferred habitats, but invertebrate prey mortality is not likely to eliminate the species' entire prey base. Because the llanero coquí is able to eat a variety of invertebrate dietary items and not all of their range is near agricultural areas, we expect the species to still have prey items available.

Toxicity Summary

Because the llanero coquí is a wetland dwelling amphibian, we do not anticipate any mortality from carbaryl exposure on-field or off-field. We do not expect individuals will forage on treated fields. We anticipate a small impact to reproduction is likely because the reproductive activity of the llanero coquí takes place on and within terrestrial habitat within the wetland (on leaf surfaces). We anticipate indirect effects are likely to occur to invertebrate prey organisms in areas within the species' range that are adjacent to agricultural areas but not throughout the range of the llanero coquí. In addition, they rely on a variety of insect prey, so we expect low levels of indirect effects from prey loss. As such, we determine the llanero coquí has a low toxicity ranking.

Overall Toxicity Ranking: Low

Effects of the Action Summary

The llanero coquí has a low exposure ranking. There is a medium extent of overlap (5.5%) between agricultural use sites and the species' range. Because the species does not occur on-field, we expect 0.96% of the range overlaps with any areas that could be exposed to insecticides, including carbaryl, in the future. With the two label restrictions above and

mitigation measures for non-agricultural uses, we anticipate that the palustrine wetland habitats of the llanero coquí will experience no more than low levels of runoff and spray drift from nearby agricultural or non-agricultural applications of carbaryl. As such, we expect a small number of individuals are likely to be exposed to carbaryl.

The llanero coquí has a low toxicity ranking. We do not expect the species to forage on-field, nor do we anticipate any llanero coquí will die on-field or off-field. We expect a low level of reproductive effects based on the EECs anticipated within the wetland habitat. We expect a low level of indirect adverse effects are likely from prey loss. Even though terrestrial arthropod mortality is anticipated, it is likely to occur only in wetland habitat located near carbaryl use areas and not throughout the llanero coquí's entire range.

Given that we expect a small number of individuals are likely to be exposed from agricultural or non-agricultural uses and adverse effects will be low, we determine the overall risk of adverse effects to the species is low.

Conclusion

The llanero coquí has a high vulnerability ranking due to its endangered status, limited distribution, small population size, and threats to the species and its habitat (e.g., contaminants, fire). Llanero coquíes are wetland obligates that occur where there is herbaceous vegetation and marshy, freshwater conditions. They are found on lands previously managed by the U.S. Navy and Commonwealth of Puerto Rico. The species' range is highly restricted and surrounded by anthropogenic land uses, including agriculture.

The species' range overlaps with a moderate portion of the action area (up to 5.5%), and we do not expect coquíes to occur on-field. The species' habitat occurs near agricultural areas where carbaryl may be used, so we considered off-field exposure from runoff or spray drift. We do not have carbaryl usage data for Puerto Rico, but we expect carbaryl has been used in Puerto Rico and 0.96% of the range could be treated with insecticides for agricultural uses. Across Puerto Rico, 20-70% of crops have been treated annually with any insecticide, suggesting the 0.96% overlap may be an overestimate as some of this area may not be treated with insecticides. We anticipate the general conservation measures above, including rain restrictions and aquatic habitat buffers, will further reduce the likelihood of exposure of the species, their prey, and their habitat. Because of the species' obligate relationship with wetlands (i.e., unlikely that it will occur on-field), non-agricultural mitigation measures (e.g., developed use methods like hand-held equipment), and low levels of anticipated toxic effects, we expect a small number of individuals will be exposed to carbaryl. We do not expect llanero coquíes to die from dietary exposure on-field or off-field, and we expect low indirect effects from prey loss because the coquí feeds on diverse insect prey that occur across the range and not only on-field where insect mortality is expected to be high.

Therefore, we anticipate a small number of individual llanero coquíes will experience reductions in invertebrate prey that lead to mortality or effects to reproduction over the duration of the proposed action. After adding the effects of the action and cumulative effects to the

environmental baseline, and in light of the status of the species, we have determined the proposed action is not expected to appreciably reduce the survival and recovery of the species in the wild. Thus, it is our biological opinion that the registration of carbaryl, as proposed, is not likely to jeopardize the continued existence of the llanero coquí.

References

- U.S. Fish and Wildlife Service. 2012. Endangered and Threatened Wildlife and Plants; Determination of Endangered Species Status for Coquí Llanero Throughout Its Range and Designation of Critical Habitat. Federal Register 77 (193): 60777-60802.
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- U.S. Fish and Wildlife Service. 2024. Coquí Llanero (*Eleutherodactylus juanariveroi*) 5-Year Status Review: Summary and Evaluation. Boquerón, Puerto Rico. 11pp.